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FORM PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		PF-0715 USN
		U.S. APPLICATION NO. (If known, see 37 CFR 1.5) TO BE ASSIGNED 10/030389
INTERNATIONAL APPLICATION NO. PCT/US00/18505	INTERNATIONAL FILING DATE 06 July 2000	PRIORITY DATE CLAIMED 06 July 1999

TITLE OF INVENTION
HUMAN IMMUNE RESPONSE MOLECULES

APPLICANT(S) FOR DO/EO/US
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Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is the **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to promptly begin national examination procedures (35 U.S.C. 371 (f)).
4. ☐ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau)
   b. ☐ has been communicated by the International Bureau.
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
   e. ☒ attached hereto Article 34 Amendment
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 16 below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.27 and 3.31 is included.
13. ☒ A FIRST preliminary amendment, as follows: Cancel in this application original claims 14, 15, 18, 20, 21, 23, 24, 26, 27, 29 - 81 before calculating the filing fee, without prejudice or disclaimer. Applicants submit that these claims were included in the application as filed in the interest of providing notice to the public of certain specific subject matter intended to be claimed, and are being canceled at this time in the interest of reducing filing costs. Applicants expressly state that these claims are not being canceled for reasons related to patentability, and are in fact fully supported by the specification as filed. Applicants expressly reserve the right to reinstate these claims or to add other claims during prosecution of this application or a continuation or divisional application. Applicants expressly do not disclaim the subject matter of any invention disclosed herein which is not set forth in the instantly filed claims.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
1) Transmittal Letter (2 pp, in duplicate)
2) Return Postcard
3) Express Mail Label No.: **EL 856 146 927 US**
4) Sequence Listing Statement

JC13 Rec'd PCT/PTO 03 JAN 2002

U.S. APPLICATION NO. (if known, see 37 CFR 1.5) TO BE ASSIGNED 10/030389	INTERNATIONAL APPLICATION NO.: PCT/US00/18505	ATTORNEY'S DOCKET NUMBER PF-0715 USN

17. ⊠ The following fees are submitted:
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5):
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO.......$1000.00
□ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO..$860.00
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO........$710.00
⊠ **International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)..........$710.00**
□ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)..................$100.00

ENTER APPROPRIATE BASIC FEE AMOUNT =				$710.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than □ 20 □ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	20 =	0	X $ 18.00	$	
Independent Claims	2 =	0	X $ 80.00	$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $270.00	$	
TOTAL OF ABOVE CALCULATIONS =				$	
□ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				$	
SUBTOTAL =				$710.00	
Processing fee of **$130.00** for furnishing the English translation later than □ 20 □ 30 months from the earliest claimed priority date (37 CFR 1492(f)). +				$	
TOTAL NATIONAL FEE =				$710.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by the appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +					
TOTAL FEES ENCLOSED =				$710.00	
				Amount to be Refunded:	$
				Charged:	$

a. □ A check in the amount of $______________ to cover the above fees is enclosed.
b. ⊠ Please charge my Deposit Account No. 09-0108 in the amount of $710.00 to cover the above fees.
c. ⊠ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 09-0108. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

INCYTE GENOMICS, INC.
3160 Porter Drive
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SIGNATURE

NAME: Diana Hamlet-Cox

REGISTRATION NUMBER: 33,302

DATE: 3 January 2002

HUMAN IMMUNE RESPONSE MOLECULES

TECHNICAL FIELD

This invention relates to nucleic acid and amino acid sequences of human immune response molecules and to the use of these sequences in the diagnosis, treatment, and prevention of autoimmune/inflammatory and cell proliferative disorders including cancer.

BACKGROUND OF THE INVENTION

The immune system responds to invading microorganisms in two major ways: antibody production and cell mediated responses. Antibodies are immunoglobulin proteins produced by B-lymphocytes (B cells) which bind to specific antigens and cause inactivation or promote destruction of the antigen by other cells. In addition to B-lymphocytes, the cellular components of the immune system include six different types of leukocytes, or white blood cells: monocytes, T lymphocytes (T cells), polymorphonuclear granulocytes (consisting of neutrophils, eosinophils, and basophils) and plasma cells. Additionally, fragments of megakaryocytes, a seventh type of white blood cell in the bone marrow, occur in large numbers in the blood as platelets. The immune system response ordinarily protects organisms against pathogens, but can produce undesirable effects such as autoimmune diseases and rejection of transplanted tissue. Immunosuppressants are used to treat autoimmune diseases such as rheumatoid arthritis and psoriasis, and to amelioriate the immune response to grafts or organ transplants.

All vertebrates have developed sophisticated and complex immune systems that provide protection from viral, bacterial, fungal, and parasitic infections. A key feature of the immune system is its ability to distinguish foreign molecules, or antigens, from "self" molecules. This ability is mediated primarily by secreted and transmembrane proteins expressed by leukocytes (white blood cells) such as lymphocytes, granulocytes, and monocytes.

Leukocyte migration plays an important role in the inflammatory response to infection. Leukocytes undergo polarization induced by stimulants, including chemokines, which cause chemoattractant receptors, integrins and other adhesion molecules, cytoskeletal proteins, and intracellular regulatory molecules to change their location within the leukocytes in a process mediated by signal transduction molecules including tyrosine kinases, lipid kinases, second messengers, and members of the Rho family of small GTPases (Sanchez-Madrid and del Pozo (1999) EMBO J. 18:501-511).

Most of the immunological proteins expressed by leukocytes belong to the immunoglobulin (Ig) superfamily, members of which contain one or more repeats of a conserved structural domain. This Ig domain is comprised of antiparallel β sheets joined by a disulfide bond in an arrangement

called the Ig fold. Members of the Ig superfamily include T-cell receptors, major histocompatibility (MHC) proteins, antibodies, and immune cell-specific surface markers such as CD4, CD8, and CD28.

Immune rejection of grafted or transplanted tissue is mediated by T cells via a direct and an indirect pathway. Direct response is mediated by polyclonal T cells directed toward a variety of antigens. The direct response usually occurs shortly after introduction of donor tissue and is sensitive to treatment by immunosuppressive drugs. The indirect response is oligoclonal and involves a few dominant antigen peptides. The indirect response is associated with chronic rejection, and is preceded by infiltration of transplanted tissue by CD4+ T cells (Benichou, G. (1999) Front. Biosci. 4:D476-480).

MHC proteins are cell surface markers that bind to and present foreign antigens to T cells. MHC molecules are classified as either class I or class II. Class I MHC molecules (MHC I) are expressed on the surface of most cells and are involved in the presentation of antigen to cytotoxic T cells. For example, a cell infected with virus will degrade intracellular viral proteins and express the protein fragments bound to MHC I molecules on the cell surface. The MHC I/antigen complex is recognized by cytotoxic T-cells which destroy the infected cell and the virus within. Class II MHC molecules are expressed primarily on specialized antigen-presenting cells of the immune system, such as B-cells and macrophages. These cells ingest foreign proteins from the extracellular fluid and express MHC II/antigen complex on the cell surface. This complex activates helper T-cells, which then secrete cytokines and other factors that stimulate the immune response. MHC molecules also play an important role in organ rejection following transplantation. Rejection occurs when the recipient's T-cells respond to foreign MHC molecules on the transplanted organ in the same way as to self MHC molecules bound to foreign antigen. (Reviewed in Alberts, B. et al. (1994) Molecular Biology of the Cell, Garland Publishing, New York, NY, pp. 1229-1246.)

Antibodies, or immunoglobulins, bind and neutralize antigens in the circulation and other extracellular fluids. Antibody classes include IgG, IgA, IgM, IgD, and IgE. IgA are found primarily in secretions and play an important role in mucosal immunity. IgA are transcytosed across epithelial cell sheets and secreted along with mucous into the lumenal space. Transcytosis and secretion are mediated by the polymeric Ig receptor, which binds to and transports IgA. The polymeric Ig receptor is a transmembrane protein with about two to five Ig domains in its extracellular domain. (Reviewed in Alberts, supra, pp. 1210-1211; Kulseth, M. A. et al. (1995) DNA Cell Biol. (1995) 14:251-256.)

Heat shock proteins (hsp) and cyclophilins, intracellular chaperone molecules that facilitate protein folding and assembly, are selectively expressed in cells following exposure to a range of stimuli including infection. Hsps are highly immunogenic, eliciting humoral, cytotoxic T lymphocyte, and natural killer cell responses against viruses, tumors, and infectious diseases. Hsp-binding immunophilins contain a tetratricopeptide repeat (TPR) present in tandem arrays of 3-16 motifs which form scaffolds to mediate protein interactions such as assembly of multiprotein complexes (Das et al.

(1998) EMBO J. 17:1192-1199).

Inhibition of cell surface molecules involved in activation can suppress the immune response. Monoclonal antibodies have been used that recognize the complex of the T cell receptor with CD3, as well as to CD5 and CD4. Side effects may include fever, gastrointestinal distress, and pulmonary edema, and immunosuppressive effect may wane. T cell activation may also be blocked by a soluble fusion protein containing a protein homologous to CD28, an adhesion receptor on the surface of most T cells that interacts with activated B cells and macrophages. Since IL-2 is critical to T cell activation and differentiation, antibodies to IL-2Rα, which is not expressed by normal resting cells, exert an immunosuppressive effect. Soluble cytokine receptors may be used as competitive cytokine receptor antagonists. TGF-β and IL-10 are cytokines that downregulate immune responses.

Immunosuppressants may intervene at a variety of stages in the immune response, such as cell-cell interaction mediated by cell surface receptors and the intracellular biochemical events involved in cellular activation and differentiation. Immunosuppressants currently available produce undesirable side effects, however. Immunosuppressants that interfere with cellular metabolism often result in significant toxicity. Agents that inhibit signal transduction at the cell surface or within the cell often compromise host defenses through nonspecific immunosuppression.

Antiproliferative immunosuppressors curtail the extensive proliferation and clonal expansion of T and B cells prerequisite to their differentiation into effector and antibody-producing cells, respectively. Antiproliferative agents that block DNA synthesis include cyclophosphamide, methotrexate, azathioprine, mizoribine, mycophenolic acid, and brequinar. They have been used to treat graft-versus-host disease, but produce side effects including granulocytopenia, azoospermia, hemorrhagic cystitis, reversible alopecia, gastrointestinal distress, and bone marrow depletion. Glucocorticosteroid hormones exert immunoregulatory effects by controlling immune and inflammatory hyperactivity during the stress response by a variety of mechanisms including disruption of leukocyte traffic, lysis of lymphocytes, and inhibition of T lymphocyte activation. Side effects produced by glucocorticosteroids may include osteoporosis, gastrointestinal distress, bone necrosis, growth suppression, and edema.

Intracellular signaling processes critical to the immune response may be inhibited by agents including cyclosporine, FK-506, and rapamycin. FK-596 and rapamycin inhibit Ca2+ associated signal transduction events involved in lymphocyte signal transduction. Rapamycin inhibits the S6 kinase stimulated by IL-2, and may alter components of the cascade regulating the response of both lymphoid and nonlymphoid cells to cytokines that promote growth. The complex of FK-506 with FK-506 Binding Protein, or the complex of cyclosporine with its binding protein cyclophilin, interacts with calcineurin, a calcium-calmodulin dependent protein phosphatase which acts to concentrate the drug in

the cell. FK-506 and rapamycin exert a stronger immunosuppressive effect than cyclosporine but can produce neurologic side effects such as headache, insomnia, paresthesias, and lethargy, as well as gastrointestinal distress and diabetes. (For a review of immunosuppression, see Paul, W.E. (1993) Fundamental Immunology, Raven Press, Ltd., New York NY pp.1-20 and 903-912).

Immunosuppressants are used to treat autoimmune diseases such as rheumatoid arthritis and psoriasis, and to ameliorate the immune response to grafts or organ transplants.

Disorders of the immune system include various autoimmune and inflammatory diseases caused by failure of the immune system to discriminate self from non-self molecules. Other immune system disorders are caused by uncontrolled cell proliferation, including leukemias such as multiple myeloma and lymphomas such as Hodgkin's disease. Immunodeficiency, brought on by a variety of diseases and agents including HIV, renders afflicted individuals susceptible to severe and sometimes fatal bacterial and viral infections. (See, for example, Golub, E. S. et al. (1987) Immunology: A Synthesis, Sinauer Associates, Sunderland, MA, pages 481 and 509-530.)

The discovery of new human immune response molecules and the polynucleotides encoding them satisfies a need in the art by providing new compositions which are useful in the diagnosis, prevention, and treatment of autoimmune/inflammatory and cell proliferative disorders including cancer.

SUMMARY OF THE INVENTION

The invention features purified polypeptides, human immune response molecules, referred to collectively as "IMUN" and individually as "IMUN-1," "IMUN-2," "IMUN-3," "IMUN-4," "IMUN-5" "IMUN-6," "IMUN-7," "IMUN-8," "IMUN-9," and "IMUN-10." In one aspect, the invention provides an isolated polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-10. In one alternative, the invention provides an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:1-10.

The invention further provides an isolated polynucleotide encoding a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, c) a biologically active fragment of an amino acid sequence selected from the group consisting of

SEQ ID NO:1-10, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-10. In one alternative, the polynucleotide encodes a polypeptide selected from the group consisting of SEQ ID NO:1-10. In another alternative, the polynucleotide is selected from the group consisting of SEQ ID NO:11-20.

Additionally, the invention provides a recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide encoding a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-10. In one alternative, the invention provides a cell transformed with the recombinant polynucleotide. In another alternative, the invention provides a transgenic organism comprising the recombinant polynucleotide.

The invention also provides a method for producing a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-10. The method comprises a) culturing a cell under conditions suitable for expression of the polypeptide, wherein said cell is transformed with a recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide encoding the polypeptide, and b) recovering the polypeptide so expressed.

Additionally, the invention provides an isolated antibody which specifically binds to a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-10.

The invention further provides an isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:11-20, b) a naturally occurring polynucleotide sequence having at least 70% sequence

identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:11-20, c) a polynucleotide sequence complementary to a), d) a polynucleotide sequence complementary to b), and e) an RNA equivalent of a)-d). In one alternative, the polynucleotide comprises at least 60 contiguous nucleotides.

Additionally, the invention provides a method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:11-20, b) a naturally occurring polynucleotide sequence having at least 70% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:11-20, c) a polynucleotide sequence complementary to a), d) a polynucleotide sequence complementary to b), and e) an RNA equivalent of a)-d). The method comprises a) hybridizing the sample with a probe comprising at least 20 contiguous nucleotides comprising a sequence complementary to said target polynucleotide in the sample, and which probe specifically hybridizes to said target polynucleotide, under conditions whereby a hybridization complex is formed between said probe and said target polynucleotide or fragments thereof, and b) detecting the presence or absence of said hybridization complex, and optionally, if present, the amount thereof. In one alternative, the probe comprises at least 60 contiguous nucleotides.

The invention further provides a method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:11-20, b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:11-20, c) a polynucleotide sequence complementary to a), d) a polynucleotide sequence complementary to b), and e) an RNA equivalent of a)-d). The method comprises a) amplifying said target polynucleotide or fragment thereof using polymerase chain reaction amplification, and b) detecting the presence or absence of said amplified target polynucleotide or fragment thereof, and, optionally, if present, the amount thereof.

The invention further provides a pharmaceutical composition comprising an effective amount of a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, and a pharmaceutically acceptable

excipient. In one embodiment, the pharmaceutical composition comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1-10. The invention additionally provides a method of treating a disease or condition associated with decreased expression of functional IMUN, comprising administering to a patient in need of such treatment the pharmaceutical composition.

The invention also provides a method for screening a compound for effectiveness as an agonist of a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-10. The method comprises a) exposing a sample comprising the polypeptide to a compound, and b) detecting agonist activity in the sample. In one alternative, the invention provides a pharmaceutical composition comprising an agonist compound identified by the method and a pharmaceutically acceptable excipient. In another alternative, the invention provides a method of treating a disease or condition associated with decreased expression of functional IMUN, comprising administering to a patient in need of such treatment the pharmaceutical composition.

Additionally, the invention provides a method for screening a compound for effectiveness as an antagonist of a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-10. The method comprises a) exposing a sample comprising the polypeptide to a compound, and b) detecting antagonist activity in the sample. In one alternative, the invention provides a pharmaceutical composition comprising an antagonist compound identified by the method and a pharmaceutically acceptable excipient. In another alternative, the invention provides a method of treating a disease or condition associated with overexpression of functional IMUN, comprising administering to a patient in need of such treatment the pharmaceutical composition.

The invention further provides a method of screening for a compound that specifically binds to a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, c) a biologically active fragment of an amino acid sequence selected

from the group consisting of SEQ ID NO:1-10, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-10. The method comprises a) combining the polypeptide with at least one test compound under suitable conditions, and b) detecting binding of the polypeptide to the test compound, thereby identifying a compound that specifically binds to the polypeptide.

The invention further provides a method of screening for a compound that modulates the activity of a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-10, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-10. The method comprises a) combining the polypeptide with at least one test compound under conditions permissive for the activity of the polypeptide, b) assessing the activity of the polypeptide in the presence of the test compound, and c) comparing the activity of the polypeptide in the presence of the test compound with the activity of the polypeptide in the absence of the test compound, wherein a change in the activity of the polypeptide in the presence of the test compound is indicative of a compound that modulates the activity of the polypeptide.

The invention further provides a method for screening a compound for effectiveness in altering expression of a target polynucleotide, wherein said target polynucleotide comprises a sequence selected from the group consisting of SEQ ID NO:11-20, the method comprising a) exposing a sample comprising the target polynucleotide to a compound, and b) detecting altered expression of the target polynucleotide.

BRIEF DESCRIPTION OF THE TABLES

Table 1 shows polypeptide and nucleotide sequence identification numbers (SEQ ID NOs), clone identification numbers (clone IDs), cDNA libraries, and cDNA fragments used to assemble full-length sequences encoding IMUN.

Table 2 shows features of each polypeptide sequence, including potential motifs, homologous sequences, and methods, algorithms, and searchable databases used for analysis of IMUN.

Table 3 shows selected fragments of each nucleic acid sequence; the tissue-specific expression patterns of each nucleic acid sequence as determined by northern analysis; diseases, disorders, or conditions associated with these tissues; and the vector into which each cDNA was cloned.

Table 4 describes the tissues used to construct the cDNA libraries from which cDNA clones encoding IMUN were isolated.

Table 5 shows the tools, programs, and algorithms used to analyze the polynucleotides and polypeptides of the invention, along with applicable descriptions, references, and threshold parameters.

DESCRIPTION OF THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular machines, materials and methods described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality of such host cells, and a reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any machines, materials, and methods similar or equivalent to those described herein can be used to practice or test the present invention, the preferred machines, materials and methods are now described. All publications mentioned herein are cited for the purpose of describing and disclosing the cell lines, protocols, reagents and vectors which are reported in the publications and which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

DEFINITIONS

"IMUN" refers to the amino acid sequences of substantially purified IMUN obtained from any species, particularly a mammalian species, including bovine, ovine, porcine, murine, equine, and human, and from any source, whether natural, synthetic, semi-synthetic, or recombinant.

The term "agonist" refers to a molecule which intensifies or mimics the biological activity of IMUN. Agonists may include proteins, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of IMUN either by directly interacting with IMUN or by acting on components of the biological pathway in which IMUN participates.

An "allelic variant" is an alternative form of the gene encoding IMUN. Allelic variants may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. A gene may have none, one, or many allelic variants of its naturally occurring form. Common mutational changes which give rise to

allelic variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

"Altered" nucleic acid sequences encoding IMUN include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a polypeptide the same as IMUN or a polypeptide with at least one functional characteristic of IMUN. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding IMUN, and improper or unexpected hybridization to allelic variants, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding IMUN. The encoded protein may also be "altered," and may contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent IMUN. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological or immunological activity of IMUN is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid, and positively charged amino acids may include lysine and arginine. Amino acids with uncharged polar side chains having similar hydrophilicity values may include: asparagine and glutamine; and serine and threonine. Amino acids with uncharged side chains having similar hydrophilicity values may include: leucine, isoleucine, and valine; glycine and alanine; and phenylalanine and tyrosine.

The terms "amino acid" and "amino acid sequence" refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. Where "amino acid sequence" is recited to refer to a sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

"Amplification" relates to the production of additional copies of a nucleic acid sequence. Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art.

The term "antagonist" refers to a molecule which inhibits or attenuates the biological activity of IMUN. Antagonists may include proteins such as antibodies, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of IMUN either by directly interacting with IMUN or by acting on components of the biological pathway in which IMUN participates.

The term "antibody" refers to intact immunoglobulin molecules as well as to fragments thereof, such as Fab, $F(ab')_2$, and Fv fragments, which are capable of binding an epitopic determinant. Antibodies that bind IMUN polypeptides can be prepared using intact polypeptides or using fragments

containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) can be derived from the translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

The term "antigenic determinant" refers to that region of a molecule (i.e., an epitope) that makes contact with a particular antibody. When a protein or a fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to antigenic determinants (particular regions or three-dimensional structures on the protein). An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

The term "antisense" refers to any composition capable of base-pairing with the "sense" (coding) strand of a specific nucleic acid sequence. Antisense compositions may include DNA; RNA; peptide nucleic acid (PNA); oligonucleotides having modified backbone linkages such as phosphorothioates, methylphosphonates, or benzylphosphonates; oligonucleotides having modified sugar groups such as 2'-methoxyethyl sugars or 2'-methoxyethoxy sugars; or oligonucleotides having modified bases such as 5-methyl cytosine, 2'-deoxyuracil, or 7-deaza-2'-deoxyguanosine. Antisense molecules may be produced by any method including chemical synthesis or transcription. Once introduced into a cell, the complementary antisense molecule base-pairs with a naturally occurring nucleic acid sequence produced by the cell to form duplexes which block either transcription or translation. The designation "negative" or "minus" can refer to the antisense strand, and the designation "positive" or "plus" can refer to the sense strand of a reference DNA molecule.

The term "biologically active" refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" or "immunogenic" refers to the capability of the natural, recombinant, or synthetic IMUN, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

"Complementary" describes the relationship between two single-stranded nucleic acid sequences that anneal by base-pairing. For example, 5'-AGT-3' pairs with its complement, 3'-TCA-5'.

A "composition comprising a given polynucleotide sequence" and a "composition comprising a given amino acid sequence" refer broadly to any composition containing the given polynucleotide or amino acid sequence. The composition may comprise a dry formulation or an aqueous solution. Compositions comprising polynucleotide sequences encoding IMUN or fragments of IMUN may be

employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing salts (e.g., NaCl), detergents (e.g., sodium dodecyl sulfate; SDS), and other components (e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.).

"Consensus sequence" refers to a nucleic acid sequence which has been subjected to repeated DNA sequence analysis to resolve uncalled bases, extended using the XL-PCR kit (PE Biosystems, Foster City CA) in the 5' and/or the 3' direction, and resequenced, or which has been assembled from one or more overlapping cDNA, EST, or genomic DNA fragments using a computer program for fragment assembly, such as the GELVIEW fragment assembly system (GCG, Madison WI) or Phrap (University of Washington, Seattle WA). Some sequences have been both extended and assembled to produce the consensus sequence.

"Conservative amino acid substitutions" are those substitutions that are predicted to least interfere with the properties of the original protein, i.e., the structure and especially the function of the protein is conserved and not significantly changed by such substitutions. The table below shows amino acids which may be substituted for an original amino acid in a protein and which are regarded as conservative amino acid substitutions.

Original Residue	Conservative Substitution
Ala	Gly, Ser
Arg	His, Lys
Asn	Asp, Gln, His
Asp	Asn, Glu
Cys	Ala, Ser
Gln	Asn, Glu, His
Glu	Asp, Gln, His
Gly	Ala
His	Asn, Arg, Gln, Glu
Ile	Leu, Val
Leu	Ile, Val
Lys	Arg, Gln, Glu
Met	Leu, Ile
Phe	His, Met, Leu, Trp, Tyr
Ser	Cys, Thr
Thr	Ser, Val
Trp	Phe, Tyr
Tyr	His, Phe, Trp
Val	Ile, Leu, Thr

Conservative amino acid substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a beta sheet or alpha helical conformation, (b) the charge or hydrophobicity of the molecule at the site of the substitution, and/or (c) the bulk of the side chain.

A "deletion" refers to a change in the amino acid or nucleotide sequence that results in the absence of one or more amino acid residues or nucleotides.

The term "derivative" refers to a chemically modified polynucleotide or polypeptide. Chemical modifications of a polynucleotide sequence can include, for example, replacement of hydrogen by an alkyl, acyl, hydroxyl, or amino group. A derivative polynucleotide encodes a polypeptide which retains at least one biological or immunological function of the natural molecule. A derivative polypeptide is one modified by glycosylation, pegylation, or any similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

A "detectable label" refers to a reporter molecule or enzyme that is capable of generating a measurable signal and is covalently or noncovalently joined to a polynucleotide or polypeptide.

A "fragment" is a unique portion of IMUN or the polynucleotide encoding IMUN which is identical in sequence to but shorter in length than the parent sequence. A fragment may comprise up to the entire length of the defined sequence, minus one nucleotide/amino acid residue. For example, a fragment may comprise from 5 to 1000 contiguous nucleotides or amino acid residues. A fragment used as a probe, primer, antigen, therapeutic molecule, or for other purposes, may be at least 5, 10, 15, 16, 20, 25, 30, 40, 50, 60, 75, 100, 150, 250 or at least 500 contiguous nucleotides or amino acid residues in length. Fragments may be preferentially selected from certain regions of a molecule. For example, a polypeptide fragment may comprise a certain length of contiguous amino acids selected from the first 250 or 500 amino acids (or first 25% or 50% of a polypeptide) as shown in a certain defined sequence. Clearly these lengths are exemplary, and any length that is supported by the specification, including the Sequence Listing, tables, and figures, may be encompassed by the present embodiments.

A fragment of SEQ ID NO:11-20 comprises a region of unique polynucleotide sequence that specifically identifies SEQ ID NO:11-20, for example, as distinct from any other sequence in the genome from which the fragment was obtained. A fragment of SEQ ID NO:11-20 is useful, for example, in hybridization and amplification technologies and in analogous methods that distinguish SEQ ID NO:11-20 from related polynucleotide sequences. The precise length of a fragment of SEQ ID NO:11-20 and the region of SEQ ID NO:11-20 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

A fragment of SEQ ID NO:1-10 is encoded by a fragment of SEQ ID NO:11-20. A fragment of SEQ ID NO:1-10 comprises a region of unique amino acid sequence that specifically identifies SEQ ID NO:1-10. For example, a fragment of SEQ ID NO:1-10 is useful as an immunogenic peptide for the development of antibodies that specifically recognize SEQ ID NO:1-10. The precise length of a fragment of SEQ ID NO:1-10 and the region of SEQ ID NO:1-10 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended

purpose for the fragment.

A "full-length" polynucleotide sequence is one containing at least a translation initiation codon (e.g., methionine) followed by an open reading frame and a translation termination codon. A "full-length" polynucleotide sequence encodes a "full-length" polypeptide sequence.

"Homology" refers to sequence similarity or, interchangeably, sequence identity, between two or more polynucleotide sequences or two or more polypeptide sequences.

The terms "percent identity" and "% identity," as applied to polynucleotide sequences, refer to the percentage of residue matches between at least two polynucleotide sequences aligned using a standardized algorithm. Such an algorithm may insert, in a standardized and reproducible way, gaps in the sequences being compared in order to optimize alignment between two sequences, and therefore achieve a more meaningful comparison of the two sequences.

Percent identity between polynucleotide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program. This program is part of the LASERGENE software package, a suite of molecular biological analysis programs (DNASTAR, Madison WI). CLUSTAL V is described in Higgins, D.G. and P.M. Sharp (1989) CABIOS 5:151-153 and in Higgins, D.G. et al. (1992) CABIOS 8:189-191. For pairwise alignments of polynucleotide sequences, the default parameters are set as follows: Ktuple=2, gap penalty=5, window=4, and "diagonals saved"=4. The "weighted" residue weight table is selected as the default. Percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polynucleotide sequences.

Alternatively, a suite of commonly used and freely available sequence comparison algorithms is provided by the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) (Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410), which is available from several sources, including the NCBI, Bethesda, MD, and on the Internet at http://www.ncbi.nlm.nih.gov/BLAST/. The BLAST software suite includes various sequence analysis programs including "blastn," that is used to align a known polynucleotide sequence with other polynucleotide sequences from a variety of databases. Also available is a tool called "BLAST 2 Sequences" that is used for direct pairwise comparison of two nucleotide sequences. "BLAST 2 Sequences" can be accessed and used interactively at http://www.ncbi.nlm.nih.gov/gorf/bl2.html. The "BLAST 2 Sequences" tool can be used for both blastn and blastp (discussed below). BLAST programs are commonly used with gap and other parameters set to default settings. For example, to compare two nucleotide sequences, one may use blastn with the "BLAST 2 Sequences" tool Version 2.0.12 (April-21-2000) set at default parameters. Such default parameters may be, for example:

Matrix: BLOSUM62

Reward for match: 1

Penalty for mismatch: -2

Open Gap: 5 and Extension Gap: 2 penalties

Gap x drop-off: 50

Expect: 10

Word Size: 11

Filter: on

Percent identity may be measured over the length of an entire defined sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined sequence, for instance, a fragment of at least 20, at least 30, at least 40, at least 50, at least 70, at least 100, or at least 200 contiguous nucleotides. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures, or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

Nucleic acid sequences that do not show a high degree of identity may nevertheless encode similar amino acid sequences due to the degeneracy of the genetic code. It is understood that changes in a nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid sequences that all encode substantially the same protein.

The phrases "percent identity" and "% identity," as applied to polypeptide sequences, refer to the percentage of residue matches between at least two polypeptide sequences aligned using a standardized algorithm. Methods of polypeptide sequence alignment are well-known. Some alignment methods take into account conservative amino acid substitutions. Such conservative substitutions, explained in more detail above, generally preserve the charge and hydrophobicity at the site of substitution, thus preserving the structure (and therefore function) of the polypeptide.

Percent identity between polypeptide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program (described and referenced above). For pairwise alignments of polypeptide sequences using CLUSTAL V, the default parameters are set as follows: Ktuple=1, gap penalty=3, window=5, and "diagonals saved"=5. The PAM250 matrix is selected as the default residue weight table. As with polynucleotide alignments, the percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polypeptide sequence pairs.

Alternatively the NCBI BLAST software suite may be used. For example, for a pairwise comparison of two polypeptide sequences, one may use the "BLAST 2 Sequences" tool Version 2.0.12 (Apr-21-2000) with blastp set at default parameters. Such default parameters may be, for example:

Matrix: BLOSUM62
Open Gap: 11 and Extension Gap: 1 penalties
Gap x drop-off: 50
Expect: 10
Word Size: 3
Filter: on

Percent identity may be measured over the length of an entire defined polypeptide sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined polypeptide sequence, for instance, a fragment of at least 15, at least 20, at least 30, at least 40, at least 50, at least 70 or at least 150 contiguous residues. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

"Human artificial chromosomes" (HACs) are linear microchromosomes which may contain DNA sequences of about 6 kb to 10 Mb in size, and which contain all of the elements required for chromosome replication, segregation and maintenance.

The term "humanized antibody" refers to an antibody molecule in which the amino acid sequence in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

"Hybridization" refers to the process by which a polynucleotide strand anneals with a complementary strand through base pairing under defined hybridization conditions. Specific hybridization is an indication that two nucleic acid sequences share a high degree of complementarity. Specific hybridization complexes form under permissive annealing conditions and remain hybridized after the "washing" step(s). The washing step(s) is particularly important in determining the stringency of the hybridization process, with more stringent conditions allowing less non-specific binding, i.e., binding between pairs of nucleic acid strands that are not perfectly matched. Permissive conditions for annealing of nucleic acid sequences are routinely determinable by one of ordinary skill in the art and may be consistent among hybridization experiments, whereas wash conditions may be varied among experiments to achieve the desired stringency, and therefore hybridization specificity. Permissive annealing conditions occur, for example, at 68°C in the presence of about 6 x SSC, about 1% (w/v) SDS, and about 100 µg/ml sheared, denatured salmon sperm DNA.

Generally, stringency of hybridization is expressed, in part, with reference to the temperature under which the wash step is carried out. Such wash temperatures are typically selected to be about 5°C to 20°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic

strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. An equation for calculating T_m and conditions for nucleic acid hybridization are well known and can be found in Sambrook, J. et al., 1989, Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; specifically see volume 2, chapter 9.

High stringency conditions for hybridization between polynucleotides of the present invention include wash conditions of 68°C in the presence of about 0.2 x SSC and about 0.1% SDS, for 1 hour. Alternatively, temperatures of about 65°C, 60°C, 55°C, or 42°C may be used. SSC concentration may be varied from about 0.1 to 2 x SSC, with SDS being present at about 0.1%. Typically, blocking reagents are used to block non-specific hybridization. Such blocking reagents include, for instance, sheared and denatured salmon sperm DNA at about 100-200 µg/ml. Organic solvent, such as formamide at a concentration of about 35-50% v/v, may also be used under particular circumstances, such as for RNA:DNA hybridizations. Useful variations on these wash conditions will be readily apparent to those of ordinary skill in the art. Hybridization, particularly under high stringency conditions, may be suggestive of evolutionary similarity between the nucleotides. Such similarity is strongly indicative of a similar role for the nucleotides and their encoded polypeptides.

The term "hybridization complex" refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary bases. A hybridization complex may be formed in solution (e.g., C_0t or R_0t analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed).

The words "insertion" and "addition" refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively.

"Immune response" can refer to conditions associated with inflammation, trauma, immune disorders, or infectious or genetic disease, etc. These conditions can be characterized by expression of various factors, e.g., cytokines, chemokines, and other signaling molecules, which may affect cellular and systemic defense systems.

An "immunogenic fragment" is a polypeptide or oligopeptide fragment of IMUN which is capable of eliciting an immune response when introduced into a living organism, for example, a mammal. The term "immunogenic fragment" also includes any polypeptide or oligopeptide fragment of IMUN which is useful in any of the antibody production methods disclosed herein or known in the art.

The term "microarray" refers to an arrangement of a plurality of polynucleotides, polypeptides, or other chemical compounds on a substrate.

The terms "element" and "array element" refer to a polynucleotide, polypeptide, or other chemical compound having a unique and defined position on a microarray.

The term "modulate" refers to a change in the activity of IMUN. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional, or immunological properties of IMUN.

The phrases "nucleic acid" and "nucleic acid sequence" refer to a nucleotide, oligonucleotide, polynucleotide, or any fragment thereof. These phrases also refer to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material.

"Operably linked" refers to the situation in which a first nucleic acid sequence is placed in a functional relationship with a second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Operably linked DNA sequences may be in close proximity or contiguous and, where necessary to join two protein coding regions, in the same reading frame.

"Peptide nucleic acid" (PNA) refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of amino acid residues ending in lysine. The terminal lysine confers solubility to the composition. PNAs preferentially bind complementary single stranded DNA or RNA and stop transcript elongation, and may be pegylated to extend their lifespan in the cell.

"Post-translational modification" of an IMUN may involve lipidation, glycosylation, phosphorylation, acetylation, racemization, proteolytic cleavage, and other modifications known in the art. These processes may occur synthetically or biochemically. Biochemical modifications will vary by cell type depending on the enzymatic milieu of IMUN.

"Probe" refers to nucleic acid sequences encoding IMUN, their complements, or fragments thereof, which are used to detect identical, allelic or related nucleic acid sequences. Probes are isolated oligonucleotides or polynucleotides attached to a detectable label or reporter molecule. Typical labels include radioactive isotopes, ligands, chemiluminescent agents, and enzymes. "Primers" are short nucleic acids, usually DNA oligonucleotides, which may be annealed to a target polynucleotide by complementary base-pairing. The primer may then be extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification (and identification) of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR).

Probes and primers as used in the present invention typically comprise at least 15 contiguous nucleotides of a known sequence. In order to enhance specificity, longer probes and primers may also be employed, such as probes and primers that comprise at least 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or at least 150 consecutive nucleotides of the disclosed nucleic acid sequences. Probes and primers may

be considerably longer than these examples, and it is understood that any length supported by the specification, including the tables, figures, and Sequence Listing, may be used.

Methods for preparing and using probes and primers are described in the references, for example Sambrook, J. et al., 1989, Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; Ausubel, F.M. et al.,1987, Current Protocols in Molecular Biology, Greene Publ. Assoc. & Wiley-Intersciences, New York NY; Innis, M. et al., 1990, PCR Protocols, A Guide to Methods and Applications, Academic Press, San Diego CA. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge MA).

Oligonucleotides for use as primers are selected using software known in the art for such purpose. For example, OLIGO 4.06 software is useful for the selection of PCR primer pairs of up to 100 nucleotides each, and for the analysis of oligonucleotides and larger polynucleotides of up to 5,000 nucleotides from an input polynucleotide sequence of up to 32 kilobases. Similar primer selection programs have incorporated additional features for expanded capabilities. For example, the PrimOU primer selection program (available to the public from the Genome Center at University of Texas South West Medical Center, Dallas TX) is capable of choosing specific primers from megabase sequences and is thus useful for designing primers on a genome-wide scope. The Primer3 primer selection program (available to the public from the Whitehead Institute/MIT Center for Genome Research, Cambridge MA) allows the user to input a "mispriming library," in which sequences to avoid as primer binding sites are user-specified. Primer3 is useful, in particular, for the selection of oligonucleotides for microarrays. (The source code for the latter two primer selection programs may also be obtained from their respective sources and modified to meet the user's specific needs.) The PrimeGen program (available to the public from the UK Human Genome Mapping Project Resource Centre, Cambridge UK) designs primers based on multiple sequence alignments, thereby allowing selection of primers that hybridize to either the most conserved or least conserved regions of aligned nucleic acid sequences. Hence, this program is useful for identification of both unique and conserved oligonucleotides and polynucleotide fragments. The oligonucleotides and polynucleotide fragments identified by any of the above selection methods are useful in hybridization technologies, for example, as PCR or sequencing primers, microarray elements, or specific probes to identify fully or partially complementary polynucleotides in a sample of nucleic acids. Methods of oligonucleotide selection are not limited to those described above.

A "recombinant nucleic acid" is a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two or more otherwise separated segments of sequence.

This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques such as those described in Sambrook, supra. The term recombinant includes nucleic acids that have been altered solely by addition, substitution, or deletion of a portion of the nucleic acid. Frequently, a recombinant nucleic acid may include a nucleic acid sequence operably linked to a promoter sequence. Such a recombinant nucleic acid may be part of a vector that is used, for example, to transform a cell.

Alternatively, such recombinant nucleic acids may be part of a viral vector, e.g., based on a vaccinia virus, that could be use to vaccinate a mammal wherein the recombinant nucleic acid is expressed, inducing a protective immunological response in the mammal.

A "regulatory element" refers to a nucleic acid sequence usually derived from untranslated regions of a gene and includes enhancers, promoters, introns, and 5' and 3' untranslated regions (UTRs). Regulatory elements interact with host or viral proteins which control transcription, translation, or RNA stability.

"Reporter molecules" are chemical or biochemical moieties used for labeling a nucleic acid, amino acid, or antibody. Reporter molecules include radionuclides; enzymes; fluorescent, chemiluminescent, or chromogenic agents; substrates; cofactors; inhibitors; magnetic particles; and other moieties known in the art.

An "RNA equivalent," in reference to a DNA sequence, is composed of the same linear sequence of nucleotides as the reference DNA sequence with the exception that all occurrences of the nitrogenous base thymine are replaced with uracil, and the sugar backbone is composed of ribose instead of deoxyribose.

The term "sample" is used in its broadest sense. A sample suspected of containing nucleic acids encoding IMUN, or fragments thereof, or IMUN itself, may comprise a bodily fluid; an extract from a cell, chromosome, organelle, or membrane isolated from a cell; a cell; genomic DNA, RNA, or cDNA, in solution or bound to a substrate; a tissue; a tissue print; etc.

The terms "specific binding" and "specifically binding" refer to that interaction between a protein or peptide and an agonist, an antibody, an antagonist, a small molecule, or any natural or synthetic binding composition. The interaction is dependent upon the presence of a particular structure of the protein, e.g., the antigenic determinant or epitope, recognized by the binding molecule. For example, if an antibody is specific for epitope "A," the presence of a polypeptide comprising the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

The term "substantially purified" refers to nucleic acid or amino acid sequences that are removed from their natural environment and are isolated or separated, and are at least 60% free,

preferably at least 75% free, and most preferably at least 90% free from other components with which they are naturally associated.

A "substitution" refers to the replacement of one or more amino acid residues or nucleotides by different amino acid residues or nucleotides, respectively.

"Substrate" refers to any suitable rigid or semi-rigid support including membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, plates, polymers, microparticles and capillaries. The substrate can have a variety of surface forms, such as wells, trenches, pins, channels and pores, to which polynucleotides or polypeptides are bound.

A "transcript image" refers to the collective pattern of gene expression by a particular cell type or tissue under given conditions at a given time.

"Transformation" describes a process by which exogenous DNA is introduced into a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation is selected based on the type of host cell being transformed and may include, but is not limited to, bacteriophage or viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term "transformed" cells includes stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome, as well as transiently transformed cells which express the inserted DNA or RNA for limited periods of time.

A "transgenic organism," as used herein, is any organism, including but not limited to animals and plants, in which one or more of the cells of the organism contains heterologous nucleic acid introduced by way of human intervention, such as by transgenic techniques well known in the art. The nucleic acid is introduced into the cell, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. The term genetic manipulation does not include classical cross-breeding, or in vitro fertilization, but rather is directed to the introduction of a recombinant DNA molecule. The transgenic organisms contemplated in accordance with the present invention include bacteria, cyanobacteria, fungi, plants, and animals. The isolated DNA of the present invention can be introduced into the host by methods known in the art, for example infection, transfection, transformation or transconjugation. Techniques for transferring the DNA of the present invention into such organisms are widely known and provided in references such as Sambrook et al. (1989), supra.

A "variant" of a particular nucleic acid sequence is defined as a nucleic acid sequence having at least 40% sequence identity to the particular nucleic acid sequence over a certain length of one of the nucleic acid sequences using blastn with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999)

set at default parameters. Such a pair of nucleic acids may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95% or at least 98% or greater sequence identity over a certain defined length. A variant may be described as, for example, an "allelic" (as defined above), "splice," "species," or "polymorphic" variant. A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternative splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or lack domains that are present in the reference molecule. Species variants are polynucleotide sequences that vary from one species to another. The resulting polypeptides generally will have significant amino acid identity relative to each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs) in which the polynucleotide sequence varies by one nucleotide base. The presence of SNPs may be indicative of, for example, a certain population, a disease state, or a propensity for a disease state.

A "variant" of a particular polypeptide sequence is defined as a polypeptide sequence having at least 40% sequence identity to the particular polypeptide sequence over a certain length of one of the polypeptide sequences using blastp with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of polypeptides may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% or greater sequence identity over a certain defined length of one of the polypeptides.

THE INVENTION

The invention is based on the discovery of new human immune response molecules (IMUN), the polynucleotides encoding IMUN, and the use of these compositions for the diagnosis, treatment, or prevention of autoimmune/inflammatory and cell proliferative disorders including cancer.

Table 1 lists the Incyte clones used to assemble full length nucleotide sequences encoding IMUN. Columns 1 and 2 show the sequence identification numbers (SEQ ID NOs) of the polypeptide and nucleotide sequences, respectively. Column 3 shows the clone IDs of the Incyte clones in which nucleic acids encoding each IMUN were identified, and column 4 shows the cDNA libraries from which these clones were isolated. Column 5 shows Incyte clones and their corresponding cDNA libraries. Clones for which cDNA libraries are not indicated were derived from pooled cDNA libraries. In some cases, GenBank sequence identifiers are also shown in column 5. The Incyte clones and GenBank cDNA sequences, where indicated, in column 5 were used to assemble the consensus nucleotide sequence of each IMUN and are useful as fragments in hybridization technologies.

The columns of Table 2 show various properties of each of the polypeptides of the invention: column 1 references the SEQ ID NO; column 2 shows the number of amino acid residues in each polypeptide; column 3 shows potential phosphorylation sites; column 4 shows potential glycosylation

sites; column 5 shows the amino acid residues comprising signature sequences and motifs; column 6 shows homologous sequences as identified by BLAST analysis; and column 7 shows analytical methods and in some cases, searchable databases to which the analytical methods were applied. The methods of column 7 were used to characterize each polypeptide through sequence homology and protein motifs.

The columns of Table 3 show the tissue-specificity and diseases, disorders, or conditions associated with nucleotide sequences encoding IMUN. The first column of Table 3 lists the nucleotide SEQ ID NOs. Column 2 lists fragments of the nucleotide sequences of column 1. These fragments are useful, for example, in hybridization or amplification technologies to identify SEQ ID NO:11-20 and to distinguish between SEQ ID NO:11-20 and related polynucleotide sequences. The polypeptides encoded by these fragments are useful, for example, as immunogenic peptides. Column 3 lists tissue categories which express IMUN as a fraction of total tissues expressing IMUN. Column 4 lists diseases, disorders, or conditions associated with those tissues expressing IMUN as a fraction of total tissues expressing IMUN. Column 5 lists the vectors used to subclone each cDNA library.

The columns of Table 4 show descriptions of the tissues used to construct the cDNA libraries from which cDNA clones encoding IMUN were isolated. Column 1 references the nucleotide SEQ ID NOs, column 2 shows the cDNA libraries from which these clones were isolated, and column 3 shows the tissue origins and other descriptive information relevant to the cDNA libraries in column 2.

SEQ ID NO:14 maps to chromosome 1 within the interval from 199.2 to 204.4 centiMorgans.

The invention also encompasses IMUN variants. A preferred IMUN variant is one which has at least about 80%, or alternatively at least about 90%, or even at least about 95% amino acid sequence identity to the IMUN amino acid sequence, and which contains at least one functional or structural characteristic of IMUN.

The invention also encompasses polynucleotides which encode IMUN. In a particular embodiment, the invention encompasses a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:11-20, which encodes IMUN. The polynucleotide sequences of SEQ ID NO:11-20, as presented in the Sequence Listing, embrace the equivalent RNA sequences, wherein occurrences of the nitrogenous base thymine are replaced with uracil, and the sugar backbone is composed of ribose instead of deoxyribose.

The invention also encompasses a variant of a polynucleotide sequence encoding IMUN. In particular, such a variant polynucleotide sequence will have at least about 70%, or alternatively at least about 85%, or even at least about 95% polynucleotide sequence identity to the polynucleotide sequence encoding IMUN. A particular aspect of the invention encompasses a variant of a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:11-20 which has at least about 70%, or alternatively at least about 85%, or even at least about 95% polynucleotide

sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:11-20. Any one of the polynucleotide variants described above can encode an amino acid sequence which contains at least one functional or structural characteristic of IMUN.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of polynucleotide sequences encoding IMUN, some bearing minimal similarity to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide sequence of naturally occurring IMUN, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode IMUN and its variants are generally capable of hybridizing to the nucleotide sequence of the naturally occurring IMUN under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding IMUN or its derivatives possessing a substantially different codon usage, e.g., inclusion of non-naturally occurring codons. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding IMUN and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences which encode IMUN and IMUN derivatives, or fragments thereof, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding IMUN or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in SEQ ID NO:11-20 and fragments thereof under various conditions of stringency. (See, e.g., Wahl, G.M. and S.L. Berger (1987) Methods Enzymol. 152:399-407; Kimmel, A.R. (1987) Methods Enzymol. 152:507-511.) Hybridization conditions, including annealing and wash conditions, are described in "Definitions."

Methods for DNA sequencing are well known in the art and may be used to practice any of the embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE (US Biochemical, Cleveland OH), Taq polymerase (PE Biosystems,

Foster City CA), thermostable T7 polymerase (Amersham Pharmacia Biotech, Piscataway NJ), or combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE amplification system (Life Technologies, Gaithersburg MD). Preferably, sequence preparation is automated with machines such as the MICROLAB 2200 liquid transfer system (Hamilton, Reno NV), PTC200 thermal cycler (MJ Research, Watertown MA) and ABI CATALYST 800 thermal cycler (PE Biosystems). Sequencing is then carried out using either the ABI 373 or 377 DNA sequencing system (PE Biosystems), the MEGABACE 1000 DNA sequencing system (Molecular Dynamics, Sunnyvale CA), or other systems known in the art. The resulting sequences are analyzed using a variety of algorithms which are well known in the art. (See, e.g., Ausubel, F.M. (1997) Short Protocols in Molecular Biology, John Wiley & Sons, New York NY, unit 7.7; Meyers, R.A. (1995) Molecular Biology and Biotechnology, Wiley VCH, New York NY, pp. 856-853.)

The nucleic acid sequences encoding IMUN may be extended utilizing a partial nucleotide sequence and employing various PCR-based methods known in the art to detect upstream sequences, such as promoters and regulatory elements. For example, one method which may be employed, restriction-site PCR, uses universal and nested primers to amplify unknown sequence from genomic DNA within a cloning vector. (See, e.g., Sarkar, G. (1993) PCR Methods Applic. 2:318-322.) Another method, inverse PCR, uses primers that extend in divergent directions to amplify unknown sequence from a circularized template. The template is derived from restriction fragments comprising a known genomic locus and surrounding sequences. (See, e.g., Triglia, T. et al. (1988) Nucleic Acids Res. 16:8186.) A third method, capture PCR, involves PCR amplification of DNA fragments adjacent to known sequences in human and yeast artificial chromosome DNA. (See, e.g., Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119.) In this method, multiple restriction enzyme digestions and ligations may be used to insert an engineered double-stranded sequence into a region of unknown sequence before performing PCR. Other methods which may be used to retrieve unknown sequences are known in the art. (See, e.g., Parker, J.D. et al. (1991) Nucleic Acids Res. 19:3055-3060). Additionally, one may use PCR, nested primers, and PROMOTERFINDER libraries (Clontech, Palo Alto CA) to walk genomic DNA. This procedure avoids the need to screen libraries and is useful in finding intron/exon junctions. For all PCR-based methods, primers may be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences, Plymouth MN) or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the template at temperatures of about 68°C to 72°C.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. In addition, random-primed libraries, which often include

sequences containing the 5' regions of genes, are preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different nucleotide-specific, laser-stimulated fluorescent dyes, and a charge coupled device camera for detection of the emitted wavelengths. Output/light intensity may be converted to electrical signal using appropriate software (e.g., GENOTYPER and SEQUENCE NAVIGATOR, PE Biosystems), and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for sequencing small DNA fragments which may be present in limited amounts in a particular sample.

In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode IMUN may be cloned in recombinant DNA molecules that direct expression of IMUN, or fragments or functional equivalents thereof, in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced and used to express IMUN.

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter IMUN-encoding sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, and so forth.

The nucleotides of the present invention may be subjected to DNA shuffling techniques such as MOLECULARBREEDING (Maxygen Inc., Santa Clara CA; described in U.S. Patent Number 5,837,458; Chang, C.-C. et al. (1999) Nat. Biotechnol. 17:793-797; Christians, F.C. et al. (1999) Nat. Biotechnol. 17:259-264; and Crameri, A. et al. (1996) Nat. Biotechnol. 14:315-319) to alter or improve the biological properties of IMUN, such as its biological or enzymatic activity or its ability to bind to other molecules or compounds. DNA shuffling is a process by which a library of gene variants is produced using PCR-mediated recombination of gene fragments. The library is then subjected to selection or screening procedures that identify those gene variants with the desired properties. These preferred variants may then be pooled and further subjected to recursive rounds of DNA shuffling and selection/screening. Thus, genetic diversity is created through "artificial" breeding and rapid molecular evolution. For example, fragments of a single gene containing random

point mutations may be recombined, screened, and then reshuffled until the desired properties are optimized. Alternatively, fragments of a given gene may be recombined with fragments of homologous genes in the same gene family, either from the same or different species, thereby maximizing the genetic diversity of multiple naturally occurring genes in a directed and controllable manner.

In another embodiment, sequences encoding IMUN may be synthesized, in whole or in part, using chemical methods well known in the art. (See, e.g., Caruthers, M.H. et al. (1980) Nucleic Acids Symp. Ser. 7:215-223; and Horn, T. et al. (1980) Nucleic Acids Symp. Ser. 7:225-232.) Alternatively, IMUN itself or a fragment thereof may be synthesized using chemical methods. For example, peptide synthesis can be performed using various solution-phase or solid-phase techniques. (See, e.g., Creighton, T. (1984) Proteins, Structures and Molecular Properties, WH Freeman, New York NY, pp. 55-60; and Roberge, J.Y. et al. (1995) Science 269:202-204.) Automated synthesis may be achieved using the ABI 431A peptide synthesizer (PE Biosystems). Additionally, the amino acid sequence of IMUN, or any part thereof, may be altered during direct synthesis and/or combined with sequences from other proteins, or any part thereof, to produce a variant polypeptide or a polypeptide having a sequence of a naturally occurring polypeptide.

The peptide may be substantially purified by preparative high performance liquid chromatography. (See, e.g., Chiez, R.M. and F.Z. Regnier (1990) Methods Enzymol. 182:392-421.) The composition of the synthetic peptides may be confirmed by amino acid analysis or by sequencing. (See, e.g., Creighton, supra, pp. 28-53.)

In order to express a biologically active IMUN, the nucleotide sequences encoding IMUN or derivatives thereof may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable host. These elements include regulatory sequences, such as enhancers, constitutive and inducible promoters, and 5' and 3' untranslated regions in the vector and in polynucleotide sequences encoding IMUN. Such elements may vary in their strength and specificity. Specific initiation signals may also be used to achieve more efficient translation of sequences encoding IMUN. Such signals include the ATG initiation codon and adjacent sequences, e.g. the Kozak sequence. In cases where sequences encoding IMUN and its initiation codon and upstream regulatory sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals including an in-frame ATG initiation codon should be provided by the vector. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate for the particular host cell system used. (See, e.g., Scharf, D. et al. (1994) Results Probl. Cell Differ.

20:125-162.)

Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding IMUN and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. (See, e.g., Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview NY, ch. 4, 8, and 16-17; Ausubel, F.M. et al. (1995) Current Protocols in Molecular Biology, John Wiley & Sons, New York NY, ch. 9, 13, and 16.)

A variety of expression vector/host systems may be utilized to contain and express sequences encoding IMUN. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); plant cell systems transformed with viral expression vectors (e.g., cauliflower mosaic virus, CaMV, or tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems. (See, e.g., Sambrook, supra; Ausubel, supra; Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509; Bitter, G.A. et al. (1987) Methods Enzymol. 153:516-544; Scorer, C.A. et al. (1994) Bio/Technology 12:181-184; Engelhard, E.K. et al. (1994) Proc. Natl. Acad. Sci. USA 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther. 7:1937-1945; Takamatsu, N. (1987) EMBO J. 6:307-311; Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105; The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196; Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. USA 81:3655-3659; and Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355.) Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population. (See, e.g., Di Nicola, M. et al. (1998) Cancer Gen. Ther. 5(6):350-356; Yu, M. et al., (1993) Proc. Natl. Acad. Sci. USA 90(13):6340-6344; Buller, R.M. et al. (1985) Nature 317(6040):813-815; McGregor, D.P. et al. (1994) Mol. Immunol. 31(3):219-226; and Verma, I.M. and N. Somia (1997) Nature 389:239-242.) The invention is not limited by the host cell employed.

In bacterial systems, a number of cloning and expression vectors may be selected depending upon the use intended for polynucleotide sequences encoding IMUN. For example, routine cloning, subcloning, and propagation of polynucleotide sequences encoding IMUN can be achieved using a multifunctional E. coli vector such as PBLUESCRIPT (Stratagene, La Jolla CA) or PSPORT1 plasmid (Life Technologies). Ligation of sequences encoding IMUN into the vector's multiple cloning site disrupts the *lacZ* gene, allowing a colorimetric screening procedure for identification of transformed bacteria containing recombinant molecules. In addition, these vectors may be useful for in vitro

transcription, dideoxy sequencing, single strand rescue with helper phage, and creation of nested deletions in the cloned sequence. (See, e.g., Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509.) When large quantities of IMUN are needed, e.g. for the production of antibodies, vectors which direct high level expression of IMUN may be used. For example, vectors containing the strong, inducible T5 or T7 bacteriophage promoter may be used.

Yeast expression systems may be used for production of IMUN. A number of vectors containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH promoters, may be used in the yeast Saccharomyces cerevisiae or Pichia pastoris. In addition, such vectors direct either the secretion or intracellular retention of expressed proteins and enable integration of foreign sequences into the host genome for stable propagation. (See, e.g., Ausubel, 1995, supra; Bitter, supra; and Scorer, supra.)

Plant systems may also be used for expression of IMUN. Transcription of sequences encoding IMUN may be driven viral promoters, e.g., the 35S and 19S promoters of CaMV used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) EMBO J. 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used. (See, e.g., Coruzzi, supra; Broglie, supra; and Winter, supra.) These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. (See, e.g., The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196.)

In mammalian cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding IMUN may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain infective virus which expresses IMUN in host cells. (See, e.g., Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. USA 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells. SV40 or EBV-based vectors may also be used for high-level protein expression.

Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained in and expressed from a plasmid. HACs of about 6 kb to 10 Mb are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355.)

For long term production of recombinant proteins in mammalian systems, stable expression of IMUN in cell lines is preferred. For example, sequences encoding IMUN can be transformed into cell lines using expression vectors which may contain viral origins of replication and/or endogenous

expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for about 1 to 2 days in enriched media before being switched to selective media. The purpose of the selectable marker is to confer resistance to a selective agent, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be propagated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase and adenine phosphoribosyltransferase genes, for use in *tk*$^-$ and *apr*$^-$ cells, respectively. (See, e.g., Wigler, M. et al. (1977) Cell 11:223-232; Lowy, I. et al. (1980) Cell 22:817-823.) Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate; *neo* confers resistance to the aminoglycosides neomycin and G-418; and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. (See, e.g., Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. USA 77:3567-3570; Colbere-Garapin, F. et al. (1981) J. Mol. Biol. 150:1-14.) Additional selectable genes have been described, e.g., *trpB* and *hisD*, which alter cellular requirements for metabolites. (See, e.g., Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. USA 85:8047-8051.) Visible markers, e.g., anthocyanins, green fluorescent proteins (GFP; Clontech), ß glucuronidase and its substrate ß-glucuronide, or luciferase and its substrate luciferin may be used. These markers can be used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system. (See, e.g., Rhodes, C.A. (1995) Methods Mol. Biol. 55:121-131.)

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, the presence and expression of the gene may need to be confirmed. For example, if the sequence encoding IMUN is inserted within a marker gene sequence, transformed cells containing sequences encoding IMUN can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding IMUN under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

In general, host cells that contain the nucleic acid sequence encoding IMUN and that express IMUN may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations, PCR amplification, and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

Immunological methods for detecting and measuring the expression of IMUN using either

specific polyclonal or monoclonal antibodies are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on IMUN is preferred, but a competitive binding assay may be employed. These and other assays are well known in the art. (See, e.g., Hampton, R. et al. (1990) Serological Methods, a Laboratory Manual, APS Press, St. Paul MN, Sect. IV; Coligan, J.E. et al. (1997) Current Protocols in Immunology, Greene Pub. Associates and Wiley-Interscience, New York NY; and Pound, J.D. (1998) Immunochemical Protocols, Humana Press, Totowa NJ.)

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding IMUN include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding IMUN, or any fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits, such as those provided by Amersham Pharmacia Biotech, Promega (Madison WI), and US Biochemical. Suitable reporter molecules or labels which may be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding IMUN may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell may be secreted or retained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode IMUN may be designed to contain signal sequences which direct secretion of IMUN through a prokaryotic or eukaryotic cell membrane.

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" or "pro" form of the protein may also be used to specify protein targeting, folding, and/or activity. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38) are available from the American Type Culture Collection (ATCC, Manassas VA) and may be chosen to ensure the correct modification and processing

of the foreign protein.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding IMUN may be ligated to a heterologous sequence resulting in translation of a fusion protein in any of the aforementioned host systems. For example, a chimeric IMUN protein containing a heterologous moiety that can be recognized by a commercially available antibody may facilitate the screening of peptide libraries for inhibitors of IMUN activity. Heterologous protein and peptide moieties may also facilitate purification of fusion proteins using commercially available affinity matrices. Such moieties include, but are not limited to, glutathione S-transferase (GST), maltose binding protein (MBP), thioredoxin (Trx), calmodulin binding peptide (CBP), 6-His, FLAG, *c-myc*, and hemagglutinin (HA). GST, MBP, Trx, CBP, and 6-His enable purification of their cognate fusion proteins on immobilized glutathione, maltose, phenylarsine oxide, calmodulin, and metal-chelate resins, respectively. FLAG, *c-myc*, and hemagglutinin (HA) enable immunoaffinity purification of fusion proteins using commercially available monoclonal and polyclonal antibodies that specifically recognize these epitope tags. A fusion protein may also be engineered to contain a proteolytic cleavage site located between the IMUN encoding sequence and the heterologous protein sequence, so that IMUN may be cleaved away from the heterologous moiety following purification. Methods for fusion protein expression and purification are discussed in Ausubel (1995, supra, ch. 10). A variety of commercially available kits may also be used to facilitate expression and purification of fusion proteins.

In a further embodiment of the invention, synthesis of radiolabeled IMUN may be achieved in vitro using the TNT rabbit reticulocyte lysate or wheat germ extract system (Promega). These systems couple transcription and translation of protein-coding sequences operably associated with the T7, T3, or SP6 promoters. Translation takes place in the presence of a radiolabeled amino acid precursor, for example, ^{35}S-methionine.

IMUN of the present invention or fragments thereof may be used to screen for compounds that specifically bind to IMUN. At least one and up to a plurality of test compounds may be screened for specific binding to IMUN. Examples of test compounds include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

In one embodiment, the compound thus identified is closely related to the natural ligand of IMUN, e.g., a ligand or fragment thereof, a natural substrate, a structural or functional mimetic, or a natural binding partner. (See, Coligan, J.E. et al. (1991) Current Protocols in Immunology 1(2): Chapter 5.) Similarly, the compound can be closely related to the natural receptor to which IMUN binds, or to at least a fragment of the receptor, e.g., the ligand binding site. In either case, the compound can be rationally designed using known techniques. In one embodiment, screening for these compounds involves producing appropriate cells which express IMUN, either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, Drosophila, or E.

coli. Cells expressing IMUN or cell membrane fractions which contain IMUN are then contacted with a test compound and binding, stimulation, or inhibition of activity of either IMUN or the compound is analyzed.

An assay may simply test binding of a test compound to the polypeptide, wherein binding is detected by a fluorophore, radioisotope, enzyme conjugate, or other detectable label. For example, the assay may comprise the steps of combining at least one test compound with IMUN, either in solution or affixed to a solid support, and detecting the binding of IMUN to the compound. Alternatively, the assay may detect or measure binding of a test compound in the presence of a labeled competitor. Additionally, the assay may be carried out using cell-free preparations, chemical libraries, or natural product mixtures, and the test compound(s) may be free in solution or affixed to a solid support.

IMUN of the present invention or fragments thereof may be used to screen for compounds that modulate the activity of IMUN. Such compounds may include agonists, antagonists, or partial or inverse agonists. In one embodiment, an assay is performed under conditions permissive for IMUN activity, wherein IMUN is combined with at least one test compound, and the activity of IMUN in the presence of a test compound is compared with the activity of IMUN in the absence of the test compound. A change in the activity of IMUN in the presence of the test compound is indicative of a compound that modulates the activity of IMUN. Alternatively, a test compound is combined with an in vitro or cell-free system comprising IMUN under conditions suitable for IMUN activity, and the assay is performed. In either of these assays, a test compound which modulates the activity of IMUN may do so indirectly and need not come in direct contact with the test compound. At least one and up to a plurality of test compounds may be screened.

In another embodiment, polynucleotides encoding IMUN or their mammalian homologs may be "knocked out" in an animal model system using homologous recombination in embryonic stem (ES) cells. Such techniques are well known in the art and are useful for the generation of animal models of human disease. (See, e.g., U.S. Patent No. 5,175,383 and U.S. Patent No. 5,767,337.) For example, mouse ES cells, such as the mouse 129/SvJ cell line, are derived from the early mouse embryo and grown in culture. The ES cells are transformed with a vector containing the gene of interest disrupted by a marker gene, e.g., the neomycin phosphotransferase gene (neo; Capecchi, M.R. (1989) Science 244:1288-1292). The vector integrates into the corresponding region of the host genome by homologous recombination. Alternatively, homologous recombination takes place using the Cre-loxP system to knockout a gene of interest in a tissue- or developmental stage-specific manner (Marth, J.D. (1996) Clin. Invest. 97:1999-2002; Wagner, K.U. et al. (1997) Nucleic Acids Res. 25:4323-4330). Transformed ES cells are identified and microinjected into mouse cell blastocysts such as those from the C57BL/6 mouse strain. The blastocysts are surgically transferred

to pseudopregnant dams, and the resulting chimeric progeny are genotyped and bred to produce heterozygous or homozygous strains. Transgenic animals thus generated may be tested with potential therapeutic or toxic agents.

Polynucleotides encoding IMUN may also be manipulated in vitro in ES cells derived from human blastocysts. Human ES cells have the potential to differentiate into at least eight separate cell lineages including endoderm, mesoderm, and ectodermal cell types. These cell lineages differentiate into, for example, neural cells, hematopoietic lineages, and cardiomyocytes (Thomson, J.A. et al. (1998) Science 282:1145-1147).

Polynucleotides encoding IMUN can also be used to create "knockin" humanized animals (pigs) or transgenic animals (mice or rats) to model human disease. With knockin technology, a region of a polynucleotide encoding IMUN is injected into animal ES cells, and the injected sequence integrates into the animal cell genome. Transformed cells are injected into blastulae, and the blastulae are implanted as described above. Transgenic progeny or inbred lines are studied and treated with potential pharmaceutical agents to obtain information on treatment of a human disease. Alternatively, a mammal inbred to overexpress IMUN, e.g., by secreting IMUN in its milk, may also serve as a convenient source of that protein (Janne, J. et al. (1998) Biotechnol. Annu. Rev. 4:55-74).

THERAPEUTICS

Chemical and structural similarity, e.g., in the context of sequences and motifs, exists between regions of IMUN and human immune response molecules. In addition, the expression of IMUN is closely associated with cell proliferation and inflammation. Therefore, IMUN appears to play a role in autoimmune/inflammatory and cell proliferative disorders including cancer. In the treatment of disorders associated with increased IMUN expression or activity, it is desirable to decrease the expression or activity of IMUN. In the treatment of disorders associated with decreased IMUN expression or activity, it is desirable to increase the expression or activity of IMUN.

Therefore, in one embodiment, IMUN or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of IMUN. Examples of such disorders include, but are not limited to, an autoimmune/inflammatory disorder such as acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or

pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma; and a cell proliferative disorder such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus.

In another embodiment, a vector capable of expressing IMUN or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of IMUN including, but not limited to, those described above.

In a further embodiment, a pharmaceutical composition comprising a substantially purified IMUN in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of IMUN including, but not limited to, those provided above.

In still another embodiment, an agonist which modulates the activity of IMUN may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of IMUN including, but not limited to, those listed above.

In a further embodiment, an antagonist of IMUN may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of IMUN. Examples of such disorders include, but are not limited to, those autoimmune/inflammatory and cell proliferative disorders including cancer described above. In one aspect, an antibody which specifically binds IMUN may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissues which express IMUN.

In an additional embodiment, a vector expressing the complement of the polynucleotide encoding IMUN may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of IMUN including, but not limited to, those described above.

In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences, or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by

one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

An antagonist of IMUN may be produced using methods which are generally known in the art. In particular, purified IMUN may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind IMUN. Antibodies to IMUN may also be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e., those which inhibit dimer formation) are generally preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with IMUN or with any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to IMUN have an amino acid sequence consisting of at least about 5 amino acids, and generally will consist of at least about 10 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein. Short stretches of IMUN amino acids may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

Monoclonal antibodies to IMUN may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (See, e.g., Kohler, G. et al. (1975) Nature 256:495-497; Kozbor, D. et al. (1985) J. Immunol. Methods 81:31-42; Cote, R.J. et al. (1983) Proc. Natl. Acad. Sci. USA 80:2026-2030; and Cole, S.P. et al. (1984) Mol. Cell Biol. 62:109-120.)

In addition, techniques developed for the production of "chimeric antibodies," such as the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used. (See, e.g., Morrison, S.L. et al. (1984) Proc. Natl. Acad. Sci. USA 81:6851-6855; Neuberger, M.S. et al. (1984) Nature 312:604-608; and Takeda,

S. et al. (1985) Nature 314:452-454.) Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce IMUN-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries. (See, e.g., Burton, D.R. (1991) Proc. Natl. Acad. Sci. USA 88:10134-10137.)

Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature. (See, e.g., Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. USA 86:3833-3837; Winter, G. et al. (1991) Nature 349:293-299.)

Antibody fragments which contain specific binding sites for IMUN may also be generated. For example, such fragments include, but are not limited to, $F(ab')_2$ fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the F(ab')2 fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (See, e.g., Huse, W.D. et al. (1989) Science 246:1275-1281.)

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between IMUN and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering IMUN epitopes is generally used, but a competitive binding assay may also be employed (Pound, supra).

Various methods such as Scatchard analysis in conjunction with radioimmunoassay techniques may be used to assess the affinity of antibodies for IMUN. Affinity is expressed as an association constant, K_a, which is defined as the molar concentration of IMUN-antibody complex divided by the molar concentrations of free antigen and free antibody under equilibrium conditions. The K_a determined for a preparation of polyclonal antibodies, which are heterogeneous in their affinities for multiple IMUN epitopes, represents the average affinity, or avidity, of the antibodies for IMUN. The K_a determined for a preparation of monoclonal antibodies, which are monospecific for a particular IMUN epitope, represents a true measure of affinity. High-affinity antibody preparations with K_a ranging from about 10^9 to 10^{12} L/mole are preferred for use in immunoassays in which the IMUN-antibody complex must withstand rigorous manipulations. Low-affinity antibody preparations with K_a ranging from about 10^6 to 10^7 L/mole are preferred for use in immunopurification and similar procedures which ultimately require dissociation of IMUN, preferably in active form, from the antibody (Catty, D. (1988)

Antibodies, Volume I: A Practical Approach, IRL Press, Washington DC; Liddell, J.E. and A. Cryer (1991) A Practical Guide to Monoclonal Antibodies, John Wiley & Sons, New York NY).

The titer and avidity of polyclonal antibody preparations may be further evaluated to determine the quality and suitability of such preparations for certain downstream applications. For example, a polyclonal antibody preparation containing at least 1-2 mg specific antibody/ml, preferably 5-10 mg specific antibody/ml, is generally employed in procedures requiring precipitation of IMUN-antibody complexes. Procedures for evaluating antibody specificity, titer, and avidity, and guidelines for antibody quality and usage in various applications, are generally available. (See, e.g., Catty, supra, and Coligan et al., supra.)

In another embodiment of the invention, the polynucleotides encoding IMUN, or any fragment or complement thereof, may be used for therapeutic purposes. In one aspect, modifications of gene expression can be achieved by designing complementary sequences or antisense molecules (DNA, RNA, PNA, or modified oligonucleotides) to the coding or regulatory regions of the gene encoding IMUN. Such technology is well known in the art, and antisense oligonucleotides or larger fragments can be designed from various locations along the coding or control regions of sequences encoding IMUN. (See, e.g., Agrawal, S., ed. (1996) Antisense Therapeutics, Humana Press Inc., Totawa NJ.)

In therapeutic use, any gene delivery system suitable for introduction of the antisense sequences into appropriate target cells can be used. Antisense sequences can be delivered intracellularly in the form of an expression plasmid which, upon transcription, produces a sequence complementary to at least a portion of the cellular sequence encoding the target protein. (See, e.g., Slater, J.E. et al. (1998) J. Allergy Clin. Immunol. 102(3):469-475; and Scanlon, K.J. et al. (1995) 9(13):1288-1296.) Antisense sequences can also be introduced intracellularly through the use of viral vectors, such as retrovirus and adeno-associated virus vectors. (See, e.g., Miller, A.D. (1990) Blood 76:271; Ausubel, supra; Uckert, W. and W. Walther (1994) Pharmacol. Ther. 63(3):323-347.) Other gene delivery mechanisms include liposome-derived systems, artificial viral envelopes, and other systems known in the art. (See, e.g., Rossi, J.J. (1995) Br. Med. Bull. 51(1):217-225; Boado, R.J. et al. (1998) J. Pharm. Sci. 87(11):1308-1315; and Morris, M.C. et al. (1997) Nucleic Acids Res. 25(14):2730-2736.)

In another embodiment of the invention, polynucleotides encoding IMUN may be used for somatic or germline gene therapy. Gene therapy may be performed to (i) correct a genetic deficiency (e.g., in the cases of severe combined immunodeficiency (SCID)-X1 disease characterized by X-linked inheritance (Cavazzana-Calvo, M. et al. (2000) Science 288:669-672), severe combined immunodeficiency syndrome associated with an inherited adenosine deaminase (ADA) deficiency (Blaese, R.M. et al. (1995) Science 270:475-480; Bordignon, C. et al. (1995) Science 270:470-475), cystic fibrosis (Zabner, J. et al. (1993) Cell 75:207-216; Crystal, R.G. et al. (1995) Hum. Gene

Therapy 6:643-666; Crystal, R.G. et al. (1995) Hum. Gene Therapy 6:667-703), thalassamias, familial hypercholesterolemia, and hemophilia resulting from Factor VIII or Factor IX deficiencies (Crystal, R.G. (1995) Science 270:404-410; Verma, I.M. and Somia, N. (1997) Nature 389:239-242)), (ii) express a conditionally lethal gene product (e.g., in the case of cancers which result from unregulated cell proliferation), or (iii) express a protein which affords protection against intracellular parasites (e.g., against human retroviruses, such as human immunodeficiency virus (HIV) (Baltimore, D. (1988) Nature 335:395-396; Poeschla, E. et al. (1996) Proc. Natl. Acad. Sci. USA. 93:11395-11399), hepatitis B or C virus (HBV, HCV); fungal parasites, such as Candida albicans and Paracoccidioides brasiliensis; and protozoan parasites such as Plasmodium falciparum and Trypanosoma cruzi). In the case where a genetic deficiency in IMUN expression or regulation causes disease, the expression of IMUN from an appropriate population of transduced cells may alleviate the clinical manifestations caused by the genetic deficiency.

In a further embodiment of the invention, diseases or disorders caused by deficiencies in IMUN are treated by constructing mammalian expression vectors encoding IMUN and introducing these vectors by mechanical means into IMUN-deficient cells. Mechanical transfer technologies for use with cells in vivo or ex vitro include (i) direct DNA microinjection into individual cells, (ii) ballistic gold particle delivery, (iii) liposome-mediated transfection, (iv) receptor-mediated gene transfer, and (v) the use of DNA transposons (Morgan, R.A. and W.F. Anderson (1993) Annu. Rev. Biochem. 62:191-217; Ivics, Z. (1997) Cell 91:501-510; Boulay, J-L. and H. Récipon (1998) Curr. Opin. Biotechnol. 9:445-450).

Expression vectors that may be effective for the expression of IMUN include, but are not limited to, the PCDNA 3.1, EPITAG, PRCCMV2, PREP, PVAX vectors (Invitrogen, Carlsbad CA), PCMV-SCRIPT, PCMV-TAG, PEGSH/PERV (Stratagene, La Jolla CA), and PTET-OFF, PTET-ON, PTRE2, PTRE2-LUC, PTK-HYG (Clontech, Palo Alto CA). IMUN may be expressed using (i) a constitutively active promoter, (e.g., from cytomegalovirus (CMV), Rous sarcoma virus (RSV), SV40 virus, thymidine kinase (TK), or β-actin genes), (ii) an inducible promoter (e.g., the tetracycline-regulated promoter (Gossen, M. and H. Bujard (1992) Proc. Natl. Acad. Sci. USA 89:5547-5551; Gossen, M. et al. (1995) Science 268:1766-1769; Rossi, F.M.V. and H.M. Blau (1998) Curr. Opin. Biotechnol. 9:451-456), commercially available in the T-REX plasmid (Invitrogen)); the ecdysone-inducible promoter (available in the plasmids PVGRXR and PIND; Invitrogen); the FK506/rapamycin inducible promoter; or the RU486/mifepristone inducible promoter (Rossi, F.M.V. and H.M. Blau, supra)), or (iii) a tissue-specific promoter or the native promoter of the endogenous gene encoding IMUN from a normal individual.

Commercially available liposome transformation kits (e.g., the PERFECT LIPID

TRANSFECTION KIT, available from Invitrogen) allow one with ordinary skill in the art to deliver polynucleotides to target cells in culture and require minimal effort to optimize experimental parameters. In the alternative, transformation is performed using the calcium phosphate method (Graham, F.L. and A.J. Eb (1973) Virology 52:456-467), or by electroporation (Neumann, E. et al. (1982) EMBO J. 1:841-845). The introduction of DNA to primary cells requires modification of these standardized mammalian transfection protocols.

In another embodiment of the invention, diseases or disorders caused by genetic defects with respect to IMUN expression are treated by constructing a retrovirus vector consisting of (i) the polynucleotide encoding IMUN under the control of an independent promoter or the retrovirus long terminal repeat (LTR) promoter, (ii) appropriate RNA packaging signals, and (iii) a Rev-responsive element (RRE) along with additional retrovirus *cis*-acting RNA sequences and coding sequences required for efficient vector propagation. Retrovirus vectors (e.g., PFB and PFBNEO) are commercially available (Stratagene) and are based on published data (Riviere, I. et al. (1995) Proc. Natl. Acad. Sci. USA 92:6733-6737), incorporated by reference herein. The vector is propagated in an appropriate vector producing cell line (VPCL) that expresses an envelope gene with a tropism for receptors on the target cells or a promiscuous envelope protein such as VSVg (Armentano, D. et al. (1987) J. Virol. 61:1647-1650; Bender, M.A. et al. (1987) J. Virol. 61:1639-1646; Adam, M.A. and A.D. Miller (1988) J. Virol. 62:3802-3806; Dull, T. et al. (1998) J. Virol. 72:8463-8471; Zufferey, R. et al. (1998) J. Virol. 72:9873-9880). U.S. Patent Number 5,910,434 to Rigg ("Method for obtaining retrovirus packaging cell lines producing high transducing efficiency retroviral supernatant") discloses a method for obtaining retrovirus packaging cell lines and is hereby incorporated by reference. Propagation of retrovirus vectors, transduction of a population of cells (e.g., CD4$^+$ T-cells), and the return of transduced cells to a patient are procedures well known to persons skilled in the art of gene therapy and have been well documented (Ranga, U. et al. (1997) J. Virol. 71:7020-7029; Bauer, G. et al. (1997) Blood 89:2259-2267; Bonyhadi, M.L. (1997) J. Virol. 71:4707-4716; Ranga, U. et al. (1998) Proc. Natl. Acad. Sci. USA 95:1201-1206; Su, L. (1997) Blood 89:2283-2290).

In the alternative, an adenovirus-based gene therapy delivery system is used to deliver polynucleotides encoding IMUN to cells which have one or more genetic abnormalities with respect to the expression of IMUN. The construction and packaging of adenovirus-based vectors are well known to those with ordinary skill in the art. Replication defective adenovirus vectors have proven to be versatile for importing genes encoding immunoregulatory proteins into intact islets in the pancreas (Csete, M.E. et al. (1995) Transplantation 27:263-268). Potentially useful adenoviral vectors are described in U.S. Patent Number 5,707,618 to Armentano ("Adenovirus vectors for gene therapy"), hereby incorporated by reference. For adenoviral vectors, see also Antinozzi, P.A. et al. (1999) Annu.

Rev. Nutr. 19:511-544; and Verma, I.M. and N. Somia (1997) Nature 18:389:239-242, both incorporated by reference herein.

In another alternative, a herpes-based, gene therapy delivery system is used to deliver polynucleotides encoding IMUN to target cells which have one or more genetic abnormalities with respect to the expression of IMUN. The use of herpes simplex virus (HSV)-based vectors may be especially valuable for introducing IMUN to cells of the central nervous system, for which HSV has a tropism. The construction and packaging of herpes-based vectors are well known to those with ordinary skill in the art. A replication-competent herpes simplex virus (HSV) type 1-based vector has been used to deliver a reporter gene to the eyes of primates (Liu, X. et al. (1999) Exp. Eye Res.169:385-395). The construction of a HSV-1 virus vector has also been disclosed in detail in U.S. Patent Number 5,804,413 to DeLuca ("Herpes simplex virus strains for gene transfer"), which is hereby incorporated by reference. U.S. Patent Number 5,804,413 teaches the use of recombinant HSV d92 which consists of a genome containing at least one exogenous gene to be transferred to a cell under the control of the appropriate promoter for purposes including human gene therapy. Also taught by this patent are the construction and use of recombinant HSV strains deleted for ICP4, ICP27 and ICP22. For HSV vectors, see also Goins, W.F. et al. (1999) J. Virol. 73:519-532 and Xu, H. et al. (1994) Dev. Biol. 163:152-161, hereby incorporated by reference. The manipulation of cloned herpesvirus sequences, the generation of recombinant virus following the transfection of multiple plasmids containing different segments of the large herpesvirus genomes, the growth and propagation of herpesvirus, and the infection of cells with herpesvirus are techniques well known to those of ordinary skill in the art.

In another alternative, an alphavirus (positive, single-stranded RNA virus) vector is used to deliver polynucleotides encoding IMUN to target cells. The biology of the prototypic alphavirus, Semliki Forest Virus (SFV), has been studied extensively and gene transfer vectors have been based on the SFV genome (Garoff, H. and K.-J. Li (1998) Curr. Opin. Biotech. 9:464-469). During alphavirus RNA replication, a subgenomic RNA is generated that normally encodes the viral capsid proteins. This subgenomic RNA replicates to higher levels than the full-length genomic RNA, resulting in the overproduction of capsid proteins relative to the viral proteins with enzymatic activity (e.g., protease and polymerase). Similarly, inserting the coding sequence for IMUN into the alphavirus genome in place of the capsid-coding region results in the production of a large number of IMUN-coding RNAs and the synthesis of high levels of IMUN in vector transduced cells. While alphavirus infection is typically associated with cell lysis within a few days, the ability to establish a persistent infection in hamster normal kidney cells (BHK-21) with a variant of Sindbis virus (SIN) indicates that the lytic replication of alphaviruses can be altered to suit the needs of the gene therapy application (Dryga, S.A.

et al. (1997) Virology 228:74-83). The wide host range of alphaviruses will allow the introduction of IMUN into a variety of cell types. The specific transduction of a subset of cells in a population may require the sorting of cells prior to transduction. The methods of manipulating infectious cDNA clones of alphaviruses, performing alphavirus cDNA and RNA transfections, and performing alphavirus infections, are well known to those with ordinary skill in the art.

Oligonucleotides derived from the transcription initiation site, e.g., between about positions -10 and +10 from the start site, may also be employed to inhibit gene expression. Similarly, inhibition can be achieved using triple helix base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature. (See, e.g., Gee, J.E. et al. (1994) in Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing, Mt. Kisco NY, pp. 163-177.) A complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. For example, engineered hammerhead motif ribozyme molecules may specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding IMUN.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, including the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides, corresponding to the region of the target gene containing the cleavage site, may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Complementary ribonucleic acid molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding IMUN. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize complementary RNA, constitutively or inducibly, can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

An additional embodiment of the invention encompasses a method for screening for a compound which is effective in altering expression of a polynucleotide encoding IMUN. Compounds which may be effective in altering expression of a specific polynucleotide may include, but are not limited to, oligonucleotides, antisense oligonucleotides, triple helix-forming oligonucleotides, transcription factors and other polypeptide transcriptional regulators, and non-macromolecular chemical entities which are capable of interacting with specific polynucleotide sequences. Effective compounds may alter polynucleotide expression by acting as either inhibitors or promoters of polynucleotide expression. Thus, in the treatment of disorders associated with increased IMUN expression or activity, a compound which specifically inhibits expression of the polynucleotide encoding IMUN may be therapeutically useful, and in the treament of disorders associated with decreased IMUN expression or activity, a compound which specifically promotes expression of the polynucleotide encoding IMUN may be therapeutically useful.

At least one, and up to a plurality, of test compounds may be screened for effectiveness in altering expression of a specific polynucleotide. A test compound may be obtained by any method commonly known in the art, including chemical modification of a compound known to be effective in altering polynucleotide expression; selection from an existing, commercially-available or proprietary library of naturally-occurring or non-natural chemical compounds; rational design of a compound based on chemical and/or structural properties of the target polynucleotide; and selection from a library of chemical compounds created combinatorially or randomly. A sample comprising a polynucleotide encoding IMUN is exposed to at least one test compound thus obtained. The sample may comprise, for example, an intact or permeabilized cell, or an in vitro cell-free or reconstituted biochemical system. Alterations in the expression of a polynucleotide encoding IMUN are assayed by any method commonly known in the art. Typically, the expression of a specific nucleotide is detected by hybridization with a probe having a nucleotide sequence complementary to the sequence of the polynucleotide encoding IMUN. The amount of hybridization may be quantified, thus forming the basis for a comparison of the expression of the polynucleotide both with and without exposure to one or more test compounds. Detection of a change in the expression of a polynucleotide exposed to a test compound indicates that the test compound is effective in altering the expression of

the polynucleotide. A screen for a compound effective in altering expression of a specific polynucleotide can be carried out, for example, using a Schizosaccharomyces pombe gene expression system (Atkins, D. et al. (1999) U.S. Patent No. 5.932,435; Arndt, G.M. et al. (2000) Nucleic Acids Res. 28:E15) or a human cell line such as HeLa cell (Clarke, M.L. et al. (2000) Biochem. Biophys. Res. Commun. 268:8-13). A particular embodiment of the present invention involves screening a combinatorial library of oligonucleotides (such as deoxyribonucleotides, ribonucleotides, peptide nucleic acids, and modified oligonucleotides) for antisense activity against a specific polynucleotide sequence (Bruice, T.W. et al. (1997) U.S. Patent No. 5,686,242; Bruice, T.W. et al. (2000) U.S. Patent No. 6,022,691).

Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections, or by polycationic amino polymers may be achieved using methods which are well known in the art. (See, e.g., Goldman, C.K. et al. (1997) Nat. Biotechnol. 15:462-466.)

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as humans, dogs, cats, cows, horses, rabbits, and monkeys.

An additional embodiment of the invention relates to the administration of a pharmaceutical composition which generally comprises an active ingredient formulated with a pharmaceutically acceptable excipient. Excipients may include, for example, sugars, starches, celluloses, gums, and proteins. Various formulations are commonly known and are thoroughly discussed in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing, Easton PA). Such pharmaceutical compositions may consist of IMUN, antibodies to IMUN, and mimetics, agonists, antagonists, or inhibitors of IMUN.

The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, pulmonary, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

Pharmaceutical compositions for pulmonary administration may be prepared in liquid or dry powder form. These compositions are generally aerosolized immediately prior to inhalation by the patient. In the case of small molecules (e.g. traditional low molecular weight organic drugs), aerosol delivery of fast-acting formulations is well-known in the art. In the case of macromolecules (e.g. larger peptides and proteins), recent developments in the field of pulmonary delivery via the alveolar region of the lung have enabled the practical delivery of drugs such as insulin to blood circulation (see, e.g.,

Patton, J.S. et al., U.S. Patent No. 5,997,848). Pulmonary delivery has the advantage of administration without needle injection, and obviates the need for potentially toxic penetration enhancers.

Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

Specialized forms of pharmaceutical compositions may be prepared for direct intracellular delivery of macromolecules comprising IMUN or fragments thereof. For example, liposome preparations containing a cell-impermeable macromolecule may promote cell fusion and intracellular delivery of the macromolecule. Alternatively, IMUN or a fragment thereof may be joined to a short cationic N-terminal portion from the HIV Tat-1 protein. Fusion proteins thus generated have been found to transduce into the cells of all tissues, including the brain, in a mouse model system (Schwarze, S.R. et al. (1999) Science 285:1569-1572).

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells, or in animal models such as mice, rats, rabbits, dogs, monkeys, or pigs. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example IMUN or fragments thereof, antibodies of IMUN, and agonists, antagonists or inhibitors of IMUN, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by calculating the ED_{50} (the dose therapeutically effective in 50% of the population) or LD_{50} (the dose lethal to 50% of the population) statistics. The dose ratio of toxic to therapeutic effects is the therapeutic index, which can be expressed as the LD_{50}/ED_{50} ratio. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used to formulate a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that includes the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, the sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy.

Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from about 0.1 μg to 100,000 μg, up to a total dose of about 1 gram, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

DIAGNOSTICS

In another embodiment, antibodies which specifically bind IMUN may be used for the diagnosis of disorders characterized by expression of IMUN, or in assays to monitor patients being treated with IMUN or agonists, antagonists, or inhibitors of IMUN. Antibodies useful for diagnostic purposes may be prepared in the same manner as described above for therapeutics. Diagnostic assays for IMUN include methods which utilize the antibody and a label to detect IMUN in human body fluids or in extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by covalent or non-covalent attachment of a reporter molecule. A wide variety of reporter molecules, several of which are described above, are known in the art and may be used.

A variety of protocols for measuring IMUN, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of IMUN expression. Normal or standard values for IMUN expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, for example, human subjects, with antibody to IMUN under conditions suitable for complex formation. The amount of standard complex formation may be quantitated by various methods, such as photometric means. Quantities of IMUN expressed in subject, control, and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding IMUN may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantify gene expression in biopsied tissues in which expression of IMUN may be correlated with disease. The diagnostic assay may be used to determine absence, presence, and excess expression of IMUN, and to monitor regulation of IMUN levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding IMUN or closely related molecules may be used to identify nucleic acid sequences which encode IMUN. The specificity of the probe, whether it is made

from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridization or amplification will determine whether the probe identifies only naturally occurring sequences encoding IMUN, allelic variants, or related sequences.

Probes may also be used for the detection of related sequences, and may have at least 50% sequence identity to any of the IMUN encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and may be derived from the sequence of SEQ ID NO:11-20 or from genomic sequences including promoters, enhancers, and introns of the IMUN gene.

Means for producing specific hybridization probes for DNAs encoding IMUN include the cloning of polynucleotide sequences encoding IMUN or IMUN derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, by radionuclides such as ^{32}P or ^{35}S, or by enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding IMUN may be used for the diagnosis of disorders associated with expression of IMUN. Examples of such disorders include, but are not limited to, an autoimmune/inflammatory disorder such as acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma; and a cell proliferative disorder such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland,

bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus. The polynucleotide sequences encoding IMUN may be used in Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and multiformat ELISA-like assays; and in microarrays utilizing fluids or tissues from patients to detect altered IMUN expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding IMUN may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences encoding IMUN may be labeled by standard methods and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantified and compared with a standard value. If the amount of signal in the patient sample is significantly altered in comparison to a control sample then the presence of altered levels of nucleotide sequences encoding IMUN in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or to monitor the treatment of an individual patient.

In order to provide a basis for the diagnosis of a disorder associated with expression of IMUN, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding IMUN, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with values from an experiment in which a known amount of a substantially purified polynucleotide is used. Standard values obtained in this manner may be compared with values obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

Once the presence of a disorder is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to determine if the level of expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to cancer, the presence of an abnormal amount of transcript (either under- or overexpressed) in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual

clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding IMUN may involve the use of PCR. These oligomers may be chemically synthesized, generated enzymatically, or produced in vitro. Oligomers will preferably contain a fragment of a polynucleotide encoding IMUN, or a fragment of a polynucleotide complementary to the polynucleotide encoding IMUN, and will be employed under optimized conditions for identification of a specific gene or condition. Oligomers may also be employed under less stringent conditions for detection or quantification of closely related DNA or RNA sequences.

In a particular aspect, oligonucleotide primers derived from the polynucleotide sequences encoding IMUN may be used to detect single nucleotide polymorphisms (SNPs). SNPs are substitutions, insertions and deletions that are a frequent cause of inherited or acquired genetic disease in humans. Methods of SNP detection include, but are not limited to, single-stranded conformation polymorphism (SSCP) and fluorescent SSCP (fSSCP) methods. In SSCP, oligonucleotide primers derived from the polynucleotide sequences encoding IMUN are used to amplify DNA using the polymerase chain reaction (PCR). The DNA may be derived, for example, from diseased or normal tissue, biopsy samples, bodily fluids, and the like. SNPs in the DNA cause differences in the secondary and tertiary structures of PCR products in single-stranded form, and these differences are detectable using gel electrophoresis in non-denaturing gels. In fSCCP, the oligonucleotide primers are fluorescently labeled, which allows detection of the amplimers in high-throughput equipment such as DNA sequencing machines. Additionally, sequence database analysis methods, termed in silico SNP (isSNP), are capable of identifying polymorphisms by comparing the sequence of individual overlapping DNA fragments which assemble into a common consensus sequence. These computer-based methods filter out sequence variations due to laboratory preparation of DNA and sequencing errors using statistical models and automated analyses of DNA sequence chromatograms. In the alternative, SNPs may be detected and characterized by mass spectrometry using, for example, the high throughput MASSARRAY system (Sequenom, Inc., San Diego CA).

Methods which may also be used to quantify the expression of IMUN include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and interpolating results from standard curves. (See, e.g., Melby, P.C. et al. (1993) J. Immunol. Methods 159:235-244; Duplaa, C. et al. (1993) Anal. Biochem. 212:229-236.) The speed of quantitation of multiple samples may be accelerated by running the assay in a high-throughput format where the oligomer or polynucleotide of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid

quantitation.

In further embodiments, oligonucleotides or longer fragments derived from any of the polynucleotide sequences described herein may be used as elements on a microarray. The microarray can be used in transcript imaging techniques which monitor the relative expression levels of large numbers of genes simultaneously as described in Seilhamer, J.J. et al., "Comparative Gene Transcript Analysis," U.S. Patent No. 5,840,484, incorporated herein by reference. The microarray may also be used to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, to monitor progression/regression of disease as a function of gene expression, and to develop and monitor the activities of therapeutic agents in the treatment of disease. In particular, this information may be used to develop a pharmacogenomic profile of a patient in order to select the most appropriate and effective treatment regimen for that patient. For example, therapeutic agents which are highly effective and display the fewest side effects may be selected for a patient based on his/her pharmacogenomic profile.

In another embodiment, antibodies specific for IMUN, or IMUN or fragments thereof may be used as elements on a microarray. The microarray may be used to monitor or measure protein-protein interactions, drug-target interactions, and gene expression profiles, as described above.

A particular embodiment relates to the use of the polynucleotides of the present invention to generate a transcript image of a tissue or cell type. A transcript image represents the global pattern of gene expression by a particular tissue or cell type. Global gene expression patterns are analyzed by quantifying the number of expressed genes and their relative abundance under given conditions and at a given time. (See Seilhamer et al., "Comparative Gene Transcript Analysis," U.S. Patent Number 5,840,484, expressly incorporated by reference herein.) Thus a transcript image may be generated by hybridizing the polynucleotides of the present invention or their complements to the totality of transcripts or reverse transcripts of a particular tissue or cell type. In one embodiment, the hybridization takes place in high-throughput format, wherein the polynucleotides of the present invention or their complements comprise a subset of a plurality of elements on a microarray. The resultant transcript image would provide a profile of gene activity.

Transcript images may be generated using transcripts isolated from tissues, cell lines, biopsies, or other biological samples. The transcript image may thus reflect gene expression in vivo, as in the case of a tissue or biopsy sample, or in vitro, as in the case of a cell line.

Transcript images which profile the expression of the polynucleotides of the present invention may also be used in conjunction with in vitro model systems and preclinical evaluation of pharmaceuticals, as well as toxicological testing of industrial and naturally-occurring environmental compounds. All compounds induce characteristic gene expression patterns, frequently termed

molecular fingerprints or toxicant signatures, which are indicative of mechanisms of action and toxicity (Nuwaysir, E.F. et al. (1999) Mol. Carcinog. 24:153-159; Steiner, S. and N.L. Anderson (2000) Toxicol. Lett. 112-113:467-471, expressly incorporated by reference herein). If a test compound has a signature similar to that of a compound with known toxicity, it is likely to share those toxic properties. These fingerprints or signatures are most useful and refined when they contain expression information from a large number of genes and gene families. Ideally, a genome-wide measurement of expression provides the highest quality signature. Even genes whose expression is not altered by any tested compounds are important as well, as the levels of expression of these genes are used to normalize the rest of the expression data. The normalization procedure is useful for comparison of expression data after treatment with different compounds. While the assignment of gene function to elements of a toxicant signature aids in interpretation of toxicity mechanisms, knowledge of gene function is not necessary for the statistical matching of signatures which leads to prediction of toxicity. (See, for example, Press Release 00-02 from the National Institute of Environmental Health Sciences, released February 29, 2000, available at http://www.niehs.nih.gov/oc/news/toxchip.htm.) Therefore, it is important and desirable in toxicological screening using toxicant signatures to include all expressed gene sequences.

In one embodiment, the toxicity of a test compound is assessed by treating a biological sample containing nucleic acids with the test compound. Nucleic acids that are expressed in the treated biological sample are hybridized with one or more probes specific to the polynucleotides of the present invention, so that transcript levels corresponding to the polynucleotides of the present invention may be quantified. The transcript levels in the treated biological sample are compared with levels in an untreated biological sample. Differences in the transcript levels between the two samples are indicative of a toxic response caused by the test compound in the treated sample.

Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g., Brennan, T.M. et al. (1995) U.S. Patent No. 5,474,796; Schena, M. et al. (1996) Proc. Natl. Acad. Sci. USA 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon, D. et al. (1995) PCT application WO95/35505; Heller, R.A. et al. (1997) Proc. Natl. Acad. Sci. USA 94:2150-2155; and Heller, M.J. et al. (1997) U.S. Patent No. 5,605,662.) Various types of microarrays are well known and thoroughly described in DNA Microarrays: A Practical Approach, M. Schena, ed. (1999) Oxford University Press, London, hereby expressly incorporated by reference.

In another embodiment of the invention, nucleic acid sequences encoding IMUN may be used to generate hybridization probes useful in mapping the naturally occurring genomic sequence. Either coding or noncoding sequences may be used, and in some instances, noncoding sequences may be preferable over coding sequences. For example, conservation of a coding sequence among members of a multi-gene family may potentially cause undesired cross hybridization during chromosomal

mapping. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to artificial chromosome constructions, e.g., human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single chromosome cDNA libraries. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355; Price, C.M. (1993) Blood Rev. 7:127-134; and Trask, B.J. (1991) Trends Genet. 7:149-154.) Once mapped, the nucleic acid sequences of the invention may be used to develop genetic linkage maps, for example, which correlate the inheritance of a disease state with the inheritance of a particular chromosome region or restriction fragment length polymorphism (RFLP). (See, e.g., Lander, E.S. and D. Botstein (1986) Proc. Natl. Acad. Sci. USA 83:7353-7357.)

Fluorescent in situ hybridization (FISH) may be correlated with other physical and genetic map data. (See, e.g., Heinz-Ulrich, et al. (1995) in Meyers, supra, pp. 965-968.) Examples of genetic map data can be found in various scientific journals or at the Online Mendelian Inheritance in Man (OMIM) World Wide Web site. Correlation between the location of the gene encoding IMUN on a physical map and a specific disorder, or a predisposition to a specific disorder, may help define the region of DNA associated with that disorder and thus may further positional cloning efforts.

In situ hybridization of chromosomal preparations and physical mapping techniques, such as linkage analysis using established chromosomal markers, may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the exact chromosomal locus is not known. This information is valuable to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the gene or genes responsible for a disease or syndrome have been crudely localized by genetic linkage to a particular genomic region, e.g., ataxia-telangiectasia to 11q22-23, any sequences mapping to that area may represent associated or regulatory genes for further investigation. (See, e.g., Gatti, R.A. et al. (1988) Nature 336:577-580.) The nucleotide sequence of the instant invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc., among normal, carrier, or affected individuals.

In another embodiment of the invention, IMUN, its catalytic or immunogenic fragments, or oligopeptides thereof can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between IMUN and the agent being tested may be measured.

Another technique for drug screening provides for high throughput screening of compounds having suitable binding affinity to the protein of interest. (See, e.g., Geysen, et al. (1984) PCT application WO84/03564.) In this method, large numbers of different small test compounds are synthesized on a solid substrate. The test compounds are reacted with IMUN, or fragments thereof, and

washed. Bound IMUN is then detected by methods well known in the art. Purified IMUN can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding IMUN specifically compete with a test compound for binding IMUN. In this manner, antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with IMUN.

In additional embodiments, the nucleotide sequences which encode IMUN may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

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The disclosures of all patents, applications, and publications mentioned above and below, in particular U.S. Ser. No. 60/142,572 and U.S. Ser. No. 60/153,170, are hereby expressly incorporated by reference.

EXAMPLES

I. Construction of cDNA Libraries

RNA was purchased from Clontech or isolated from tissues described in Table 4. Some tissues were homogenized and lysed in guanidinium isothiocyanate, while others were homogenized and lysed in phenol or in a suitable mixture of denaturants, such as TRIZOL (Life Technologies), a monophasic solution of phenol and guanidine isothiocyanate. The resulting lysates were centrifuged over CsCl cushions or extracted with chloroform. RNA was precipitated from the lysates with either isopropanol or sodium acetate and ethanol, or by other routine methods.

Phenol extraction and precipitation of RNA were repeated as necessary to increase RNA purity. In some cases, RNA was treated with DNase. For most libraries, poly(A+) RNA was isolated using oligo d(T)-coupled paramagnetic particles (Promega), OLIGOTEX latex particles (QIAGEN, Chatsworth CA), or an OLIGOTEX mRNA purification kit (QIAGEN). Alternatively, RNA was

isolated directly from tissue lysates using other RNA isolation kits, e.g., the POLY(A)PURE mRNA purification kit (Ambion, Austin TX).

In some cases, Stratagene was provided with RNA and constructed the corresponding cDNA libraries. Otherwise, cDNA was synthesized and cDNA libraries were constructed with the UNIZAP vector system (Stratagene) or SUPERSCRIPT plasmid system (Life Technologies), using the recommended procedures or similar methods known in the art. (See, e.g., Ausubel, 1997, supra, units 5.1-6.6.) Reverse transcription was initiated using oligo d(T) or random primers. Synthetic oligonucleotide adapters were ligated to double stranded cDNA, and the cDNA was digested with the appropriate restriction enzyme or enzymes. For most libraries, the cDNA was size-selected (300-1000 bp) using SEPHACRYL S1000, SEPHAROSE CL2B, or SEPHAROSE CL4B column chromatography (Amersham Pharmacia Biotech) or preparative agarose gel electrophoresis. cDNAs were ligated into compatible restriction enzyme sites of the polylinker of a suitable plasmid, e.g., PBLUESCRIPT plasmid (Stratagene), PSPORT1 plasmid (Life Technologies), pcDNA2.1 plasmid (Invitrogen, Carlsbad CA), or pINCY plasmid (Incyte Genomics, Palo Alto CA). Recombinant plasmids were transformed into competent E. coli cells including XL1-Blue, XL1-BlueMRF, or SOLR from Stratagene or DH5α, DH10B, or ElectroMAX DH10B from Life Technologies.

II. Isolation of cDNA Clones

Plasmids obtained as described in Example I were recovered from host cells by in vivo excision using the UNIZAP vector system (Stratagene) or by cell lysis. Plasmids were purified using at least one of the following: a Magic or WIZARD Minipreps DNA purification system (Promega); an AGTC Miniprep purification kit (Edge Biosystems, Gaithersburg MD); and QIAWELL 8 Plasmid, QIAWELL 8 Plus Plasmid, QIAWELL 8 Ultra Plasmid purification systems or the R.E.A.L. PREP 96 plasmid purification kit from QIAGEN. Following precipitation, plasmids were resuspended in 0.1 ml of distilled water and stored, with or without lyophilization, at 4°C.

Alternatively, plasmid DNA was amplified from host cell lysates using direct link PCR in a high-throughput format (Rao, V.B. (1994) Anal. Biochem. 216:1-14). Host cell lysis and thermal cycling steps were carried out in a single reaction mixture. Samples were processed and stored in 384-well plates, and the concentration of amplified plasmid DNA was quantified fluorometrically using PICOGREEN dye (Molecular Probes, Eugene OR) and a FLUOROSKAN II fluorescence scanner (Labsystems Oy, Helsinki, Finland).

III. Sequencing and Analysis

Incyte cDNA recovered in plasmids as described in Example II were sequenced as follows. Sequencing reactions were processed using standard methods or high-throughput instrumentation such as the ABI CATALYST 800 (PE Biosystems) thermal cycler or the PTC-200 thermal cycler (MJ

Research) in conjunction with the HYDRA microdispenser (Robbins Scientific) or the MICROLAB 2200 (Hamilton) liquid transfer system. cDNA sequencing reactions were prepared using reagents provided by Amersham Pharmacia Biotech or supplied in ABI sequencing kits such as the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (PE Biosystems). Electrophoretic separation of cDNA sequencing reactions and detection of labeled polynucleotides were carried out using the MEGABACE 1000 DNA sequencing system (Molecular Dynamics); the ABI PRISM 373 or 377 sequencing system (PE Biosystems) in conjunction with standard ABI protocols and base calling software; or other sequence analysis systems known in the art. Reading frames within the cDNA sequences were identified using standard methods (reviewed in Ausubel, 1997, supra, unit 7.7). Some of the cDNA sequences were selected for extension using the techniques disclosed in Example VI.

The polynucleotide sequences derived from cDNA sequencing were assembled and analyzed using a combination of software programs which utilize algorithms well known to those skilled in the art. Table 5 summarizes the tools, programs, and algorithms used and provides applicable descriptions, references, and threshold parameters. The first column of Table 5 shows the tools, programs, and algorithms used, the second column provides brief descriptions thereof, the third column presents appropriate references, all of which are incorporated by reference herein in their entirety, and the fourth column presents, where applicable, the scores, probability values, and other parameters used to evaluate the strength of a match between two sequences (the higher the score, the greater the homology between two sequences). Sequences were analyzed using MACDNASIS PRO software (Hitachi Software Engineering, South San Francisco CA) and LASERGENE software (DNASTAR). Polynucleotide and polypeptide sequence alignments were generated using the default parameters specified by the clustal algorithm as incorporated into the MEGALIGN multisequence alignment program (DNASTAR), which also calculates the percent identity between aligned sequences.

The polynucleotide sequences were validated by removing vector, linker, and polyA sequences and by masking ambiguous bases, using algorithms and programs based on BLAST, dynamic programing, and dinucleotide nearest neighbor analysis. The sequences were then queried against a selection of public databases such as the GenBank primate, rodent, mammalian, vertebrate, and eukaryote databases, and BLOCKS, PRINTS, DOMO, PRODOM, and PFAM to acquire annotation using programs based on BLAST, FASTA, and BLIMPS. The sequences were assembled into full length polynucleotide sequences using programs based on Phred, Phrap, and Consed, and were screened for open reading frames using programs based on GeneMark, BLAST, and FASTA. The full length polynucleotide sequences were translated to derive the corresponding full length amino acid sequences, and these full length sequences were subsequently analyzed by querying against databases such as the GenBank databases (described above), SwissProt, BLOCKS, PRINTS, DOMO, PRODOM, Prosite,

and Hidden Markov Model (HMM)-based protein family databases such as PFAM. HMM is a probabilistic approach which analyzes consensus primary structures of gene families. (See, e.g., Eddy, S.R. (1996) Curr. Opin. Struct. Biol. 6:361-365.)

The programs described above for the assembly and analysis of full length polynucleotide and amino acid sequences were also used to identify polynucleotide sequence fragments from SEQ ID NO:11-20. Fragments from about 20 to about 4000 nucleotides which are useful in hybridization and amplification technologies were described in The Invention section above.

IV. Analysis of Polynucleotide Expression

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound. (See, e.g., Sambrook, supra, ch. 7; Ausubel, 1995, supra, ch. 4 and 16.)

Analogous computer techniques applying BLAST were used to search for identical or related molecules in cDNA databases such as GenBank or LIFESEQ (Incyte Genomics). This analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or similar. The basis of the search is the product score, which is defined as:

$$\frac{\text{BLAST Score} \times \text{Percent Identity}}{5 \times \text{minimum}\{\text{length(Seq. 1)}, \text{length(Seq. 2)}\}}$$

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. The product score is a normalized value between 0 and 100, and is calculated as follows: the BLAST score is multiplied by the percent nucleotide identity and the product is divided by (5 times the length of the shorter of the two sequences). The BLAST score is calculated by assigning a score of +5 for every base that matches in a high-scoring segment pair (HSP), and -4 for every mismatch. Two sequences may share more than one HSP (separated by gaps). If there is more than one HSP, then the pair with the highest BLAST score is used to calculate the product score. The product score represents a balance between fractional overlap and quality in a BLAST alignment. For example, a product score of 100 is produced only for 100% identity over the entire length of the shorter of the two sequences being compared. A product score of 70 is produced either by 100% identity and 70% overlap at one end, or by 88% identity and 100% overlap at the other. A product score of 50 is produced either by 100% identity and 50% overlap at one end, or 79% identity and 100% overlap.

The results of northern analyses are reported as a percentage distribution of libraries in which the transcript encoding IMUN occurred. Analysis involved the categorization of cDNA libraries by

organ/tissue and disease. The organ/tissue categories included cardiovascular, dermatologic, developmental, endocrine, gastrointestinal, hematopoietic/immune, musculoskeletal, nervous, reproductive, and urologic. The disease/condition categories included cancer, inflammation, trauma, cell proliferation, neurological, and pooled. For each category, the number of libraries expressing the sequence of interest was counted and divided by the total number of libraries across all categories. Percentage values of tissue-specific and disease- or condition-specific expression are reported in Table 3.

V. Chromosomal Mapping of IMUN Encoding Polynucleotides

The cDNA sequences which were used to assemble SEQ ID NO:14-20 were compared with sequences from the Incyte LIFESEQ database and public domain databases using BLAST and other implementations of the Smith-Waterman algorithm. Sequences from these databases that matched SEQ ID NO:14-20 were assembled into clusters of contiguous and overlapping sequences using assembly algorithms such as Phrap (Table 5). Radiation hybrid and genetic mapping data available from public resources such as the Stanford Human Genome Center (SHGC), Whitehead Institute for Genome Research (WIGR), and Généthon were used to determine if any of the clustered sequences had been previously mapped. Inclusion of a mapped sequence in a cluster resulted in the assignment of all sequences of that cluster, including its particular SEQ ID NO, to that map location.

The genetic map location of SEQ ID NO:14 is described in The Invention as a range, or interval, of a human chromosome. The map position of an interval, in centiMorgans, is measured relative to the terminus of the chromosome's p-arm. (The centiMorgan (cM) is a unit of measurement based on recombination frequencies between chromosomal markers. On average, 1 cM is roughly equivalent to 1 megabase (Mb) of DNA in humans, although this can vary widely due to hot and cold spots of recombination.) The cM distances are based on genetic markers mapped by Généthon which provide boundaries for radiation hybrid markers whose sequences were included in each of the clusters.

VI. Extension of IMUN Encoding Polynucleotides

The full length nucleic acid sequences of SEQ ID NO:11-20 were produced by extension of an appropriate fragment of the full length molecule using oligonucleotide primers designed from this fragment. One primer was synthesized to initiate 5' extension of the known fragment, and the other primer, to initiate 3' extension of the known fragment. The initial primers were designed using OLIGO 4.06 software (National Biosciences), or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

Selected human cDNA libraries were used to extend the sequence. If more than one extension

was necessary or desired, additional or nested sets of primers were designed.

High fidelity amplification was obtained by PCR using methods well known in the art. PCR was performed in 96-well plates using the PTC-200 thermal cycler (MJ Research, Inc.). The reaction mix contained DNA template, 200 nmol of each primer, reaction buffer containing Mg^{2+}, $(NH_4)_2SO_4$, and β-mercaptoethanol, Taq DNA polymerase (Amersham Pharmacia Biotech), ELONGASE enzyme (Life Technologies), and Pfu DNA polymerase (Stratagene), with the following parameters for primer pair PCI A and PCI B: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C. In the alternative, the parameters for primer pair T7 and SK+ were as follows: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 57°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C.

The concentration of DNA in each well was determined by dispensing 100 µl PICOGREEN quantitation reagent (0.25% (v/v) PICOGREEN; Molecular Probes, Eugene OR) dissolved in 1X TE and 0.5 µl of undiluted PCR product into each well of an opaque fluorimeter plate (Corning Costar, Acton MA), allowing the DNA to bind to the reagent. The plate was scanned in a Fluoroskan II (Labsystems Oy, Helsinki, Finland) to measure the fluorescence of the sample and to quantify the concentration of DNA. A 5 µl to 10 µl aliquot of the reaction mixture was analyzed by electrophoresis on a 1 % agarose mini-gel to determine which reactions were successful in extending the sequence.

The extended nucleotides were desalted and concentrated, transferred to 384-well plates, digested with CviJI cholera virus endonuclease (Molecular Biology Research, Madison WI), and sonicated or sheared prior to religation into pUC 18 vector (Amersham Pharmacia Biotech). For shotgun sequencing, the digested nucleotides were separated on low concentration (0.6 to 0.8%) agarose gels, fragments were excised, and agar digested with Agar ACE (Promega). Extended clones were religated using T4 ligase (New England Biolabs, Beverly MA) into pUC 18 vector (Amersham Pharmacia Biotech), treated with Pfu DNA polymerase (Stratagene) to fill-in restriction site overhangs, and transfected into competent E. coli cells. Transformed cells were selected on antibiotic-containing media, and individual colonies were picked and cultured overnight at 37°C in 384-well plates in LB/2x carb liquid media.

The cells were lysed, and DNA was amplified by PCR using Taq DNA polymerase (Amersham Pharmacia Biotech) and Pfu DNA polymerase (Stratagene) with the following parameters: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 72°C, 2 min; Step 5: steps 2, 3, and 4 repeated 29 times; Step 6: 72°C, 5 min; Step 7: storage at 4°C. DNA was quantified by PICOGREEN reagent (Molecular Probes) as described above. Samples with low DNA recoveries were reamplified using the same conditions as described above. Samples were diluted with 20%

dimethysulfoxide (1:2, v/v), and sequenced using DYENAMIC energy transfer sequencing primers and the DYENAMIC DIRECT kit (Amersham Pharmacia Biotech) or the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (PE Biosystems).

In like manner, the polynucleotide sequences of SEQ ID NO:11-20 are used to obtain 5' regulatory sequences using the procedure above, along with oligonucleotides designed for such extension, and an appropriate genomic library.

VII. Labeling and Use of Individual Hybridization Probes

Hybridization probes derived from SEQ ID NO:11-20 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base pairs, is specifically described, essentially the same procedure is used with larger nucleotide fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 software (National Biosciences) and labeled by combining 50 pmol of each oligomer, 250 μCi of [γ-^{32}P] adenosine triphosphate (Amersham Pharmacia Biotech), and T4 polynucleotide kinase (DuPont NEN, Boston MA). The labeled oligonucleotides are substantially purified using a SEPHADEX G-25 superfine size exclusion dextran bead column (Amersham Pharmacia Biotech). An aliquot containing 10^7 counts per minute of the labeled probe is used in a typical membrane-based hybridization analysis of human genomic DNA digested with one of the following endonucleases: Ase I, Bgl II, Eco RI, Pst I, Xba I, or Pvu II (DuPont NEN).

The DNA from each digest is fractionated on a 0.7% agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under conditions of up to, for example, 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. Hybridization patterns are visualized using autoradiography or an alternative imaging means and compared.

VIII. Microarrays

The linkage or synthesis of array elements upon a microarray can be achieved utilizing photolithography, piezoelectric printing (ink-jet printing, See, e.g., Baldeschweiler, supra), mechanical microspotting technologies, and derivatives thereof. The substrate in each of the aforementioned technologies should be uniform and solid with a non-porous surface (Schena (1999), supra). Suggested substrates include silicon, silica, glass slides, glass chips, and silicon wafers. Alternatively, a procedure analogous to a dot or slot blot may also be used to arrange and link elements to the surface of a substrate using thermal, UV, chemical, or mechanical bonding procedures. A typical array may be produced using available methods and machines well known to those of ordinary skill in the art and may contain any appropriate number of elements. (See, e.g., Schena, M. et al. (1995) Science 270:467-470;

Shalon, D. et al. (1996) Genome Res. 6:639-645; Marshall, A. and J. Hodgson (1998) Nat. Biotechnol. 16:27-31.)

Full length cDNAs, Expressed Sequence Tags (ESTs), or fragments or oligomers thereof may comprise the elements of the microarray. Fragments or oligomers suitable for hybridization can be selected using software well known in the art such as LASERGENE software (DNASTAR). The array elements are hybridized with polynucleotides in a biological sample. The polynucleotides in the biological sample are conjugated to a fluorescent label or other molecular tag for ease of detection. After hybridization, nonhybridized nucleotides from the biological sample are removed, and a fluorescence scanner is used to detect hybridization at each array element. Alternatively, laser desorbtion and mass spectrometry may be used for detection of hybridization. The degree of complementarity and the relative abundance of each polynucleotide which hybridizes to an element on the microarray may be assessed. In one embodiment, microarray preparation and usage is described in detail below.

Tissue or Cell Sample Preparation

Total RNA is isolated from tissue samples using the guanidinium thiocyanate method and poly(A)$^+$ RNA is purified using the oligo-(dT) cellulose method. Each poly(A)$^+$ RNA sample is reverse transcribed using MMLV reverse-transcriptase, 0.05 pg/µl oligo-(dT) primer (21mer), 1X first strand buffer, 0.03 units/µl RNase inhibitor, 500 µM dATP, 500 µM dGTP, 500 µM dTTP, 40 µM dCTP, 40 µM dCTP-Cy3 (BDS) or dCTP-Cy5 (Amersham Pharmacia Biotech). The reverse transcription reaction is performed in a 25 ml volume containing 200 ng poly(A)$^+$ RNA with GEMBRIGHT kits (Incyte). Specific control poly(A)$^+$ RNAs are synthesized by in vitro transcription from non-coding yeast genomic DNA. After incubation at 37°C for 2 hr, each reaction sample (one with Cy3 and another with Cy5 labeling) is treated with 2.5 ml of 0.5M sodium hydroxide and incubated for 20 minutes at 85°C to the stop the reaction and degrade the RNA. Samples are purified using two successive CHROMA SPIN 30 gel filtration spin columns (CLONTECH Laboratories, Inc. (CLONTECH), Palo Alto CA) and after combining, both reaction samples are ethanol precipitated using 1 ml of glycogen (1 mg/ml), 60 ml sodium acetate, and 300 ml of 100% ethanol. The sample is then dried to completion using a SpeedVAC (Savant Instruments Inc., Holbrook NY) and resuspended in 14 µl 5X SSC/0.2% SDS.

Microarray Preparation

Sequences of the present invention are used to generate array elements. Each array element is amplified from bacterial cells containing vectors with cloned cDNA inserts. PCR amplification uses primers complementary to the vector sequences flanking the cDNA insert. Array elements are amplified in thirty cycles of PCR from an initial quantity of 1-2 ng to a final quantity greater than 5 µg. Amplified array elements are then purified using SEPHACRYL-400 (Amersham Pharmacia

Biotech).

Purified array elements are immobilized on polymer-coated glass slides. Glass microscope slides (Corning) are cleaned by ultrasound in 0.1% SDS and acetone, with extensive distilled water washes between and after treatments. Glass slides are etched in 4% hydrofluoric acid (VWR Scientific Products Corporation (VWR), West Chester PA), washed extensively in distilled water, and coated with 0.05% aminopropyl silane (Sigma) in 95% ethanol. Coated slides are cured in a 110°C oven.

Array elements are applied to the coated glass substrate using a procedure described in US Patent No. 5,807,522, incorporated herein by reference. 1 µl of the array element DNA, at an average concentration of 100 ng/µl, is loaded into the open capillary printing element by a high-speed robotic apparatus. The apparatus then deposits about 5 nl of array element sample per slide.

Microarrays are UV-crosslinked using a STRATALINKER UV-crosslinker (Stratagene). Microarrays are washed at room temperature once in 0.2% SDS and three times in distilled water. Non-specific binding sites are blocked by incubation of microarrays in 0.2% casein in phosphate buffered saline (PBS) (Tropix, Inc., Bedford MA) for 30 minutes at 60°C followed by washes in 0.2% SDS and distilled water as before.

Hybridization

Hybridization reactions contain 9 µl of sample mixture consisting of 0.2 µg each of Cy3 and Cy5 labeled cDNA synthesis products in 5X SSC, 0.2% SDS hybridization buffer. The sample mixture is heated to 65°C for 5 minutes and is aliquoted onto the microarray surface and covered with an 1.8 cm^2 coverslip. The arrays are transferred to a waterproof chamber having a cavity just slightly larger than a microscope slide. The chamber is kept at 100% humidity internally by the addition of 140 µl of 5X SSC in a corner of the chamber. The chamber containing the arrays is incubated for about 6.5 hours at 60°C. The arrays are washed for 10 min at 45°C in a first wash buffer (1X SSC, 0.1% SDS), three times for 10 minutes each at 45°C in a second wash buffer (0.1X SSC), and dried.

Detection

Reporter-labeled hybridization complexes are detected with a microscope equipped with an Innova 70 mixed gas 10 W laser (Coherent, Inc., Santa Clara CA) capable of generating spectral lines at 488 nm for excitation of Cy3 and at 632 nm for excitation of Cy5. The excitation laser light is focused on the array using a 20X microscope objective (Nikon, Inc., Melville NY). The slide containing the array is placed on a computer-controlled X-Y stage on the microscope and raster-scanned past the objective. The 1.8 cm x 1.8 cm array used in the present example is scanned with a resolution of 20 micrometers.

In two separate scans, a mixed gas multiline laser excites the two fluorophores sequentially. Emitted light is split, based on wavelength, into two photomultiplier tube detectors (PMT R1477,

Hamamatsu Photonics Systems, Bridgewater NJ) corresponding to the two fluorophores. Appropriate filters positioned between the array and the photomultiplier tubes are used to filter the signals. The emission maxima of the fluorophores used are 565 nm for Cy3 and 650 nm for Cy5. Each array is typically scanned twice, one scan per fluorophore using the appropriate filters at the laser source, although the apparatus is capable of recording the spectra from both fluorophores simultaneously.

The sensitivity of the scans is typically calibrated using the signal intensity generated by a cDNA control species added to the sample mixture at a known concentration. A specific location on the array contains a complementary DNA sequence, allowing the intensity of the signal at that location to be correlated with a weight ratio of hybridizing species of 1:100,000. When two samples from different sources (e.g., representing test and control cells), each labeled with a different fluorophore, are hybridized to a single array for the purpose of identifying genes that are differentially expressed, the calibration is done by labeling samples of the calibrating cDNA with the two fluorophores and adding identical amounts of each to the hybridization mixture.

The output of the photomultiplier tube is digitized using a 12-bit RTI-835H analog-to-digital (A/D) conversion board (Analog Devices, Inc., Norwood MA) installed in an IBM-compatible PC computer. The digitized data are displayed as an image where the signal intensity is mapped using a linear 20-color transformation to a pseudocolor scale ranging from blue (low signal) to red (high signal). The data is also analyzed quantitatively. Where two different fluorophores are excited and measured simultaneously, the data are first corrected for optical crosstalk (due to overlapping emission spectra) between the fluorophores using each fluorophore's emission spectrum.

A grid is superimposed over the fluorescence signal image such that the signal from each spot is centered in each element of the grid. The fluorescence signal within each element is then integrated to obtain a numerical value corresponding to the average intensity of the signal. The software used for signal analysis is the GEMTOOLS gene expression analysis program (Incyte).

IX. Complementary Polynucleotides

Sequences complementary to the IMUN-encoding sequences, or any parts thereof, are used to detect, decrease, or inhibit expression of naturally occurring IMUN. Although use of oligonucleotides comprising from about 15 to 30 base pairs is described, essentially the same procedure is used with smaller or with larger sequence fragments. Appropriate oligonucleotides are designed using OLIGO 4.06 software (National Biosciences) and the coding sequence of IMUN. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal binding to the IMUN-encoding transcript.

X. Expression of IMUN

Expression and purification of IMUN is achieved using bacterial or virus-based expression

systems. For expression of IMUN in bacteria, cDNA is subcloned into an appropriate vector containing an antibiotic resistance gene and an inducible promoter that directs high levels of cDNA transcription. Examples of such promoters include, but are not limited to, the *trp-lac* (*tac*) hybrid promoter and the T5 or T7 bacteriophage promoter in conjunction with the *lac* operator regulatory element. Recombinant vectors are transformed into suitable bacterial hosts, e.g., BL21(DE3). Antibiotic resistant bacteria express IMUN upon induction with isopropyl beta-D-thiogalactopyranoside (IPTG). Expression of IMUN in eukaryotic cells is achieved by infecting insect or mammalian cell lines with recombinant Autographica californica nuclear polyhedrosis virus (AcMNPV), commonly known as baculovirus. The nonessential polyhedrin gene of baculovirus is replaced with cDNA encoding IMUN by either homologous recombination or bacterial-mediated transposition involving transfer plasmid intermediates. Viral infectivity is maintained and the strong polyhedrin promoter drives high levels of cDNA transcription. Recombinant baculovirus is used to infect Spodoptera frugiperda (Sf9) insect cells in most cases, or human hepatocytes, in some cases. Infection of the latter requires additional genetic modifications to baculovirus. (See Engelhard, E.K. et al. (1994) Proc. Natl. Acad. Sci. USA 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther. 7:1937-1945.)

In most expression systems, IMUN is synthesized as a fusion protein with, e.g., glutathione S-transferase (GST) or a peptide epitope tag, such as FLAG or 6-His, permitting rapid, single-step, affinity-based purification of recombinant fusion protein from crude cell lysates. GST, a 26-kilodalton enzyme from Schistosoma japonicum, enables the purification of fusion proteins on immobilized glutathione under conditions that maintain protein activity and antigenicity (Amersham Pharmacia Biotech). Following purification, the GST moiety can be proteolytically cleaved from IMUN at specifically engineered sites. FLAG, an 8-amino acid peptide, enables immunoaffinity purification using commercially available monoclonal and polyclonal anti-FLAG antibodies (Eastman Kodak). 6-His, a stretch of six consecutive histidine residues, enables purification on metal-chelate resins (QIAGEN). Methods for protein expression and purification are discussed in Ausubel (1995, supra, ch. 10 and 16). Purified IMUN obtained by these methods can be used directly in the assays shown in Examples XI and XV.

XI. Demonstration of IMUN Activity

IMUN activity is represented by that of immunoglobulins, which recognize and precipitate antigens from serum. The quantitative precipitin reaction measures this activity. (Golub, E. S. et al. (1987) Immunology: A Synthesis, Sinauer Associates, Sunderland, MA, pages 113-115.) IMUN is isotopically labeled using methods known in the art. Various serum concentrations are added to constant amounts of labeled IMUN. IMUN-antigen complexes precipitate out of solution and are

collected by centrifugation. The amount of precipitable IMUN-antigen complex is proportional to the amount of radioisotope detected in the precipitate. The amount of precipitable IMUN-antigen complex is plotted against the serum concentration. For various serum concentrations, a characteristic precipitin curve is obtained, in which the amount of precipitable IMUN-antigen complex initially increases proportionately with increasing serum concentration, peaks at the equivalence point, and then decreases proportionately with further increases in serum concentration. Thus, the amount of precipitable IMUN-antigen complex is a measure of IMUN activity which is characterized by sensitivity to both limiting and excess quantities of antigen.

Alternatively, leukocytes transformed with a vector containing SEQ ID NO:11-20 are assayed for IMUN activity by measuring their response to cytokine stimulation. In this assay, the amount of tritiated thymidine incorporated into newly synthesized DNA is used to estimate proliferative activity. Varying amounts of cytokine are added to cultured leukocytes, such as granulocytes, monocytes, or lymphocytes, in the presence of [^{3}H]thymidine, a radioactive DNA precursor. Incorporation of [^{3}H]thymidine into acid-precipitable DNA is measured over an appropriate time interval, and the amount incorporated is directly proportional to the amount of newly synthesized DNA. IMUN activity is demonstrated by decreased proliferation or differentiation of transformed cytokine-stimulated leukocytes relative to untransformed cytokine-stimulated leukocytes.

XII. Functional Assays

IMUN function is assessed by expressing the sequences encoding IMUN at physiologically elevated levels in mammalian cell culture systems. cDNA is subcloned into a mammalian expression vector containing a strong promoter that drives high levels of cDNA expression. Vectors of choice include pCMV SPORT plasmid (Life Technologies) and pCR3.1 plasmid (Invitrogen), both of which contain the cytomegalovirus promoter. 5-10 μg of recombinant vector are transiently transfected into a human cell line, for example, an endothelial or hematopoietic cell line, using either liposome formulations or electroporation. 1-2 μg of an additional plasmid containing sequences encoding a marker protein are co-transfected. Expression of a marker protein provides a means to distinguish transfected cells from nontransfected cells and is a reliable predictor of cDNA expression from the recombinant vector. Marker proteins of choice include, e.g., Green Fluorescent Protein (GFP; Clontech), CD64, or a CD64-GFP fusion protein. Flow cytometry (FCM), an automated, laser optics-based technique, is used to identify transfected cells expressing GFP or CD64-GFP and to evaluate the apoptotic state of the cells and other cellular properties. FCM detects and quantifies the uptake of fluorescent molecules that diagnose events preceding or coincident with cell death. These events include changes in nuclear DNA content as measured by staining of DNA with propidium iodide; changes in cell size and granularity as measured by forward light scatter and 90 degree side light scatter; down-regulation of DNA synthesis as measured by decrease in bromodeoxyuridine uptake; alterations in

expression of cell surface and intracellular proteins as measured by reactivity with specific antibodies; and alterations in plasma membrane composition as measured by the binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in flow cytometry are discussed in Ormerod, M.G. (1994) Flow Cytometry, Oxford, New York NY.

The influence of IMUN on gene expression can be assessed using highly purified populations of cells transfected with sequences encoding IMUN and either CD64 or CD64-GFP. CD64 and CD64-GFP are expressed on the surface of transfected cells and bind to conserved regions of human immunoglobulin G (IgG). Transfected cells are efficiently separated from nontransfected cells using magnetic beads coated with either human IgG or antibody against CD64 (DYNAL, Lake Success NY). mRNA can be purified from the cells using methods well known by those of skill in the art. Expression of mRNA encoding IMUN and other genes of interest can be analyzed by northern analysis or microarray techniques.

XIII. Production of IMUN Specific Antibodies

IMUN substantially purified using polyacrylamide gel electrophoresis (PAGE; see, e.g., Harrington, M.G. (1990) Methods Enzymol. 182:488-495), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols.

Alternatively, the IMUN amino acid sequence is analyzed using LASERGENE software (DNASTAR) to determine regions of high immunogenicity, and a corresponding oligopeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Methods for selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions are well described in the art. (See, e.g., Ausubel, 1995, supra, ch. 11.)

Typically, oligopeptides of about 15 residues in length are synthesized using an ABI 431A peptide synthesizer (PE Biosystems) using FMOC chemistry and coupled to KLH (Sigma-Aldrich, St. Louis MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) to increase immunogenicity. (See, e.g., Ausubel, 1995, supra.) Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. Resulting antisera are tested for antipeptide and anti-IMUN activity by, for example, binding the peptide or IMUN to a substrate, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio-iodinated goat anti-rabbit IgG.

XIV. Purification of Naturally Occurring IMUN Using Specific Antibodies

Naturally occurring or recombinant IMUN is substantially purified by immunoaffinity chromatography using antibodies specific for IMUN. An immunoaffinity column is constructed by covalently coupling anti-IMUN antibody to an activated chromatographic resin, such as CNBr-activated SEPHAROSE (Amersham Pharmacia Biotech). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing IMUN are passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of IMUN (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/IMUN binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such as urea or thiocyanate ion), and IMUN is collected.

XV. Identification of Molecules Which Interact with IMUN

IMUN, or biologically active fragments thereof, are labeled with ^{125}I Bolton-Hunter reagent. (See, e.g., Bolton A.E. and W.M. Hunter (1973) Biochem. J. 133:529-539.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled IMUN, washed, and any wells with labeled IMUN complex are assayed. Data obtained using different concentrations of IMUN are used to calculate values for the number, affinity, and association of IMUN with the candidate molecules.

Alternatively, molecules interacting with IMUN are analyzed using the yeast two-hybrid system as described in Fields, S. and O. Song (1989, Nature 340:245-246), or using commercially available kits based on the two-hybrid system, such as the MATCHMAKER system (Clontech).

IMUN may also be used in the PATHCALLING process (CuraGen Corp., New Haven CT) which employs the yeast two-hybrid system in a high-throughput manner to determine all interactions between the proteins encoded by two large libraries of genes (Nandabalan, K. et al. (2000) U.S. Patent No. 6,057,101).

Various modifications and variations of the described methods and systems of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with certain embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

Table 1

Protein SEQ ID NO:	Nucleotide SEQ ID NO:	Clone ID	Library	Fragments
1	11	089071	LIVRNOT01	089071H1 (LIVRNOT01), 779758R1 (MYOMNOT01), 1339765H1 (COLNTUT03), 1730595F6 (BRSTTUT08), 1890182T6 (BLADTUT07), 2836509X305B1 (TLYMNOT03), 3034442F6 (TLYMNOT05), 3133921H1 (SMCCNOT01), SBDA03267F1, SBDA04709F1, SBFA03530F1, SBFA00259F1, SBFA00011F1
2	12	2378306	ISLTNOT01	014671R6 (THP1PLB01), 631453R6 (KIDNNOT05), 2137148F6 (ENDCNOT01), 2328181R6 (COLNNOT11), 2378306H1 (ISLTNOT01)
3	13	3706232	PENCNOT07	1446163H1 (PLACNOT02), 2478166F6 (SMCANOT01), 3706232H1 (PENCNOT07)
4	14	259836	HNT2RAT01	259836H1 (HNT2RAT01), 259836R6 (HNT2RAT01), 636722H1 (NEUTGMT01), 1283601F6 (COLNNOT16), 1450544F1 (PENITUT01), 1817004F6 (PROSNOT20), 1856284F6 (PROSNOT18), 2832077T6 (TLYMNOT03), 3079642H1 (BRAIUNT01)
5	15	893681	BRSTNOT05	893681H1 (BRSTNOT05), 2478039T6 (SMCANOT01), 2496565F6 (ADRETUT05), 4990588H1 (LIVRTUT11), SXAF01680V1
6	16	2109468	BRAITUT03	134614R1 (BMARNOT02), 1503436T1 (BRAITUT07), 2109468H1 (BRAITUT03), 4005589F6 (ENDCNOT04)
7	17	2223341	LUNGNOT18	1554034F6 (BLADTUT04), 2223341H1 (LUNGNOT18), 2737090F6 (OVARNOT09), SCBA05428V1, g2063041, g1042469
8	18	2291926	BRAINON01	662629R6 (BRAINOT03), 980475T6 (TONGTUT01), 1242716H1 (LUNGNOT03), 2291926H1 (BRAINON01), 3076146T6 (BONEUNT01), 3470014H1 (BRAIDIT01), g696892, g2070864
9	19	2362126	LUNGFET05	2362126H1 (LUNGFET05), 2362126R6 (LUNGFET05), g752159
10	20	5325511	FIBPFEN06	222028R6 (PANCNOT01), 756727R1 (BRAITUT02), 815931R6 (OVARTUT01), 967211R1 (BRSTNOT05), 967211T1 (BRSTNOT05), 1269450T1 (BRAINOT09), 1342796F6 (COLNTUT03), 2782127F6 (OVARTUT03), 3325542H1 (PTHYNOT03), 5325511H1 (FIBPFEN06)

Table 2

Polypeptide SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequence(s)	Homologous Sequence	Analytical Methods and Databases
1 (089071)	737	S71 S79 S259 S348 T387 S670 T710 T711 T53 T54 S79 S86 T106 T107 S137 T148 T232 T243 S286 S318 T332 S347 S451 T474 S514 S534 S18 S62 S94 S116 S125 S225 S263 S266 S272 S291 S334 S343 S344 S351 S455 T499 S502 S602 T683 S702 Y374	N214 N217 N264 N267 N280 N500	RNP-1: S348-G491 Tumor recognition sequence/ cyclophilin-type cis-trans isomerase: H249-R404	matrin cyclophilin g2828710	Motifs BLAST-GenBank BLAST-DOMO
2 (2378306)	319	S117 T34 S122 S123 T178 T189 T221 T222 S239 T266 S281 T76 T174 S205 T244 S292	N202 N220 N234 N238 N277 N296		FKBP12 interacting protein g3859944	Motifs BLAST-GenBank
3 (3706232)	131	T66		Signal peptide: M1-A20 FKBP-type isomerase: E26-G115	FK506-binding protein g2827255	Motifs BLAST-GenBank BLAST-Pfam BLAST-DOMO HMMER SPSCAN PROFILESCAN

Table 2 (cont.)

Polypeptide SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequence(s)	Homologous Sequence	Analytical Methods and Databases
4 (259836)	642	S63 S127 S165 S205 S212 S246 S431 S438 S574 T582 S136 S284 S294 T330 T403 T451 S594	N279 N299 N536 N591	L510-S634: Scruin (actin crosslinking sperm protein) domain F9-I206: POZ protein interaction domain	g4650844 Kelch motif containing protein	Motifs BLAST_GENBANK BLAST_PRODOM BLAST_DOMO
5 (893681)	349	T8 T155 S204 S77			g1020307 FK506 binding protein 51	Motifs BLAST_GENBANK
6 (2109468)	300	S174 T77 T105 T132 S141 S174 S242 S8 S19 T77 S170 T173		G15-S22: ATP/GTP-binding site motif (loop A) I11-K172: AIG1 protein	g4538998 AIG1 (Arabidopsis infection induced gene) protein	Motifs BLAST_GENBANK BLAST_PRODOM
7 (2223341)	118	S28 T88 S89 S95 S115		E6-L111: ATP-binding myosin intermediate	g2039368 circulating cathodic antigen	Motifs BLAST_GENBANK BLAST_PRODOM
8 (2291926)	313	T81 T150 T172 T177 T249 S105 T128 S202 Y135		M1-A18: Signal peptide F83-T128: Immuno-globulin superfamily		Motifs HMMER SPSCAN HMMER_PFAM BLIMPS_DOMO
9 (2362126)	139	S90 S129 S115 S118		M1-A23: Signal peptide	g1515302 retrovirus restriction polypeptide	Motifs BLAST_GENBANK SPSCAN

Table 2 (cont.)

Polypeptide SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequence(s)	Homologous Sequence	Analytical Methods and Databases
10 (5325511)	565	S322 S100 S157 S242 S388 T429 S523 T528 S539 S548 T79 T134 S179 S318 T436 Y279	N299 N471	L239-P506: TPR protein interaction repeat	g5081315 prediabetic NOD sera-reactive autoantigen g6180015 [fl][Homo sapiens] anaphase-promoting complex subunit 7	Motifs BLAST_GENBANK BLAST_DOMO

Table 3

Nucleotide SEQ ID NO:	Selected Fragment	Tissue Expression (Fraction of Total)	Disease or Condition (Fraction of Total)	Vector
11	759-803	Reproductive (0.281) Nervous (0.175) Gastrointestinal (0.140) Hematopoietic/Immune (0.140)	Cancer (0.474) Cell proliferation (0.193) Inflammation (0.210)	PBLUESCRIPT
12	273-317	Reproductive (0.283) Musculoskeletal (0.130) Hematopoietic/Immune (0.130)	Cancer (0.500) Inflammation (0.326) Cell proliferation (0.217)	pINCY
13	524-568	Cardiovascular (0.286) Reproductive (0.286) Endocrine (0.143) Musculoskeletal (0.143) Nervous (0.143)	Cancer (0.286) Cell proliferation (0.286) Inflammation (0.286)	pINCY
14	434-478	Nervous (0.195) Reproductive (0.195) Gastrointestinal (0.188)	Cancer (0.451) Inflammation (0.353) Cell proliferation(0.165)	PBLUESCRIPT
15	813-857	Reproductive (0.300) Gastrointestinal (0.200) Nervous (0.167)	Cancer (0.433) Inflammation (0.267) Cell proliferation (0.133)	PSPORT1
16	217-261	Cardiovascular (0.169) Nervous (0.169) Hematopoietic/Immune (0.154)	Inflammation (0.670) Cancer (0.308)	PSPORT1
17	207-251	Cardiovascular (0.208) Reproductive (0.250) Endocrine (0.125) Hematopoietic/Immune (0.125) Urologic (0.125)	Cancer (0.292) Inflammation (0.250) Cell proliferation (0.208)	pINCY
18	325-369	Nervous (0.625) Cardiovascular (0.125) Endocrine (0.125) Hematopoietic/Immune (0.125)	Cancer (0.375) Cell proliferation (0.125) Inflammation (0.375)	PSPORT1
19	219-263	Developmental (0.600) Gastrointestinal (0.200) Nervous (0.200)	Cell proliferation (0.600) Cancer (0.400)	PSPORT1
20	109-153	Reproductive (0.275) Nervous (0.198) Gastrointestinal (0.165)	Cancer (0.418) Inflammation (0.396) Cell proliferation (0.165)	pINCY

Table 4

Nucleotide SEQ ID NO:	Library	Library Comment
11	LIVRNOT01	Library was constructed at Stratagene, using RNA isolated from the liver tissue of a 49-year-old male.
12	ISLTNOT01	Library was constructed using RNA isolated from a pooled collection of pancreatic islet cells.
13	PENCNOT07	Library was constructed using RNA isolated from penis right corpora cavernosa tissue.
14	HNT2RAT01	Library was constructed at Stratagene (STR937231), using RNA isolated from the hNT2 cell line (derived from a human teratocarcinoma that exhibited properties characteristic of a committed neuronal precursor). Cells were treated with retinoic acid for 24 hours.
15	BRSTNOT05	Library was constructed using RNA isolated from breast tissue removed from a 58-year-old Caucasian female during a unilateral extended simple mastectomy. Pathology for the associated tumor tissue indicated multicentric invasive grade 4 lobular carcinoma. Patient history included skin cancer, rheumatic heart disease, osteoarthritis, and tuberculosis. Family history included cerebrovascular and cardiovascular disease, breast and prostate cancer, and type I diabetes.
16	BRAITUT03	Library was constructed using RNA isolated from brain tumor tissue removed from the left frontal lobe a 17-year-old Caucasian female during excision of a cerebral meningeal lesion. Pathology indicated a grade 4 fibrillary giant and small-cell astrocytoma. Family history included benign hypertension and cerebrovascular disease.
17	LUNGNOT18	Library was constructed using RNA isolated from left upper lobe lung tissue removed from a 66-year-old Caucasian female. Pathology for the associated tumor tissue indicated a grade 2 adenocarcinoma. Patient history included cerebrovascular disease, atherosclerotic coronary artery disease, and pulmonary insufficiency. Family history included a myocardial infarction and atherosclerotic coronary artery disease.
18	BRAINON01	Library was constructed and normalized from 4.88 million independent clones from a brain library. RNA was made from brain tissue removed from a 26-year-old Caucasian male during cranioplasty and excision of a cerebral meningeal lesion. Pathology for the associated tumor tissue indicated a grade 4 oligoastrocytoma in the right fronto-parietal part of the brain.
19	LUNGFET05	Library was constructed using RNA isolated from lung tissue removed from a Caucasian female fetus, who died at 20 weeks' gestation from anencephalus.

Table 4 (cont.)

Nucleotide SEQ ID NO:	Library	Library Comment
20	FIBPFEN06	The normalized prostate stromal fibroblast tissue libraries were constructed from 1.56 million independent clones from a fibroblast library. Starting RNA was made from fibroblasts of prostate stroma removed from a male fetus, who died after 26 weeks' gestation. The libraries were normalized in two rounds using conditions adapted from Soares et al., PNAS (1994) 91:9228 and Bonaldo et al.,Genome Research 6 (1996):791, except that a significantly longer (48-hours/round) reannealing hybridization was used. The library was then linearized and recircularized to select for insert containing clones as follows: plasmid DNA was prepped from approximately 1 million clones from the normalized prostate stromal fibroblast tissue libraries following soft agar transformation. The DNA was linearized with NotI and insert containing clones were size-selected by agarose gel electrophoresis and then recircularized by ligation.

What is claimed is:

1. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

a) an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10.

b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10,

c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, and

d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10.

2. An isolated polypeptide of claim 1 selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10.

3. An isolated polynucleotide encoding a polypeptide of claim 1.

4. An isolated polynucleotide encoding a polypeptide of claim 2.

5. An isolated polynucleotide of claim 4 selected from the group consisting of SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20.

6. A recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide of claim 3.

7. A cell transformed with a recombinant polynucleotide of claim 6.

8. A transgenic organism comprising a recombinant polynucleotide of claim 6.

9. A method for producing a polypeptide of claim 1, the method comprising:

a) culturing a cell under conditions suitable for expression of the polypeptide, wherein said cell is transformed with a recombinant polynucleotide, and said recombinant polynucleotide comprises a promoter sequence operably linked to a polynucleotide encoding the polypeptide of claim 1, and

b) recovering the polypeptide so expressed.

10. An isolated antibody which specifically binds to a polypeptide of claim 1.

11. An isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of:

a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20,

b) a naturally occurring polynucleotide sequence having at least 70% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20,

c) a polynucleotide sequence complementary to a),

d) a polynucleotide sequence complementary to b), and

e) an RNA equivalent of a)-d).

12. An isolated polynucleotide comprising at least 60 contiguous nucleotides of a polynucleotide of claim 11.

13. A method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide of claim 11, the method comprising:

a) hybridizing the sample with a probe comprising at least 20 contiguous nucleotides comprising a sequence complementary to said target polynucleotide in the sample, and which probe specifically hybridizes to said target polynucleotide, under conditions whereby a hybridization complex is formed between said probe and said target polynucleotide or fragments thereof, and

b) detecting the presence or absence of said hybridization complex, and, optionally, if present, the amount thereof.

14. A method of claim 13, wherein the probe comprises at least 60 contiguous nucleotides.

15. A method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide of claim 11, the method comprising:

a) amplifying said target polynucleotide or fragment thereof using polymerase chain reaction amplification, and

b) detecting the presence or absence of said amplified target polynucleotide or fragment thereof, and, optionally, if present, the amount thereof.

16. A pharmaceutical composition comprising an effective amount of a polypeptide of claim 1 and a pharmaceutically acceptable excipient.

17. A pharmaceutical composition of claim 16, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10.

18. A method for treating a disease or condition associated with decreased expression of functional IMUN, comprising administering to a patient in need of such treatment the pharmaceutical composition of claim 16.

19. A method for screening a compound for effectiveness as an agonist of a polypeptide of claim 1, the method comprising:

a) exposing a sample comprising a polypeptide of claim 1 to a compound, and

b) detecting agonist activity in the sample.

20. A pharmaceutical composition comprising an agonist compound identified by a method of claim 19 and a pharmaceutically acceptable excipient.

21. A method for treating a disease or condition associated with decreased expression of functional IMUN, comprising administering to a patient in need of such treatment a pharmaceutical composition of claim 20.

22. A method for screening a compound for effectiveness as an antagonist of a polypeptide of claim 1, the method comprising:

a) exposing a sample comprising a polypeptide of claim 1 to a compound, and

b) detecting antagonist activity in the sample.

23. A pharmaceutical composition comprising an antagonist compound identified by a method of claim 22 and a pharmaceutically acceptable excipient.

24. A method for treating a disease or condition associated with overexpression of functional IMUN, comprising administering to a patient in need of such treatment a pharmaceutical composition of claim 23.

25. A method of screening for a compound that specifically binds to the polypeptide of claim 1, said method comprising the steps of:

a) combining the polypeptide of claim 1 with at least one test compound under suitable conditions, and

b) detecting binding of the polypeptide of claim 1 to the test compound, thereby identifying a compound that specifically binds to the polypeptide of claim 1.

26. A method of screening for a compound that modulates the activity of the polypeptide of claim 1, said method comprising:

a) combining the polypeptide of claim 1 with at least one test compound under conditions permissive for the activity of the polypeptide of claim 1,

b) assessing the activity of the polypeptide of claim 1 in the presence of the test compound, and

c) comparing the activity of the polypeptide of claim 1 in the presence of the test compound with the activity of the polypeptide of claim 1 in the absence of the test compound, wherein a change in the activity of the polypeptide of claim 1 in the presence of the test compound is indicative of a compound that modulates the activity of the polypeptide of claim 1.

27. A method for screening a compound for effectiveness in altering expression of a target polynucleotide, wherein said target polynucleotide comprises a sequence of claim 5, the method comprising:

a) exposing a sample comprising the target polynucleotide to a compound, and

b) detecting altered expression of the target polynucleotide.

28. A method for assessing toxicity of a test compound, said method comprising:

a) treating a biological sample containing nucleic acids with the test compound;

b) hybridizing the nucleic acids of the treated biological sample with a probe comprising at least 20 contiguous nucleotides of a polynucleotide of claim 11 under conditions whereby a specific

hybridization complex is formed between said probe and a target polynucleotide in the biological sample, said target polynucleotide comprising a polynucleotide sequence of a polynucleotide of claim 11 or fragment thereof;

c) quantifying the amount of hybridization complex; and

d) comparing the amount of hybridization complex in the treated biological sample with the amount of hybridization complex in an untreated biological sample, wherein a difference in the amount of hybridization complex in the treated biological sample is indicative of toxicity of the test compound.

29. A method for screening a compound for effectiveness in altering expression of a target polynucleotide, wherein said target polynucleotide comprises a sequence of claim 5, the method comprising:

a) exposing a sample comprising the target polynucleotide to a compound, under conditions suitable for the expression of the target polynucleotide,

b) detecting altered expression of the target polynucleotide, and

c) comparing the expression of the target polynucleotide in the presence of varying amounts of the compound and in the absence of the compound.

30. A method of claim 9, wherein the polypeptide has the sequence of SEQ ID NO:1.

31. A method of claim 9, wherein the polypeptide has the sequence of SEQ ID NO:2.

32. A method of claim 9, wherein the polypeptide has the sequence of SEQ ID NO:3.

33. A method of claim 9, wherein the polypeptide has the sequence of SEQ ID NO:5.

34. A method of claim 9, wherein the polypeptide has the sequence of SEQ ID NO:6.

35. A method of claim 9, wherein the polypeptide has the sequence of SEQ ID NO:7.

36. A method of claim 9, wherein the polypeptide has the sequence of SEQ ID NO:8.

37. A method of claim 9, wherein the polypeptide has the sequence of SEQ ID NO:9.

38. A method of claim 9, wherein the polypeptide has the sequence of SEQ ID NO:10.

39. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:1.

40. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:2.

41. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:3.

42. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:5.

43. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:6.

44. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:7.

45. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:8.

46. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:9.

47. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:10.

48. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID NO:11.

49. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID NO:12.

50. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID NO:13.

51. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID NO:15.

52. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID NO:16.

53. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID NO:17.

54. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID NO:18.

55. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID NO:19.

56. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID NO:20.

57. A diagnostic test for a condition or disease associated with the expression of human immune response molecules (IMUN) in a biological sample comprising the steps of:

a) combining the biological sample with an antibody of claim 10, under conditions suitable for the antibody to bind the polypeptide and form an antibody:polypeptide complex; and

b) detecting the complex, wherein the presence of the complex correlates with the presence of the polypeptide in the biological sample.

58. The antibody of claim 10, wherein the antibody is:

a) a chimeric antibody,

b) a single chain antibody,

c) a Fab fragment,

d) a $F(ab')_2$ fragment, or

e) a humanized antibody.

59. A composition comprising an antibody of claim 10 and an acceptable excipient.

60. A method of diagnosing a condition or disease associated with the expression of human immune response molecules (IMUN) in a subject, comprising administering to said subject an effective amount of the composition of claim 59.

61. A composition of claim 59, wherein the antibody is labeled.

62. A method of diagnosing a condition or disease associated with the expression of human immune response molecules (IMUN) in a subject, comprising administering to said subject an effective amount of the composition of claim 61.

63. A method of preparing a polyclonal antibody with the specificity of the antibody of claim 10 comprising:

a) immunizing an animal with a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO: 3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 and SEQ ID NO:10, or an immunogenic fragment thereof, under conditions to elicit an antibody response;

b) isolating antibodies from said animal; and

c) screening the isolated antibodies with the polypeptide, thereby identifying a polyclonal antibody which binds specifically to a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO: 3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 and SEQ ID NO:10.

64. An antibody produced by a method of claim 63.

65. A composition comprising the antibody of claim 64 and a suitable carrier.

66. A method of making a monoclonal antibody with the specificity of the antibody of claim 10 comprising:

a) immunizing an animal with a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO: 3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 and SEQ ID NO:10, or an immunogenic fragment thereof, under conditions to elicit an antibody response;

b) isolating antibody producing cells from the animal;

c) fusing the antibody producing cells with immortalized cells to form monoclonal antibody-producing hybridoma cells;

d) culturing the hybridoma cells; and

e) isolating from the culture monoclonal antibody which binds specifically to a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO: 3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 and SEQ ID NO:10.

67. A monoclonal antibody produced by a method of claim 66.

68. A composition comprising the antibody of claim 67 and a suitable carrier.

69. The antibody of claim 10, wherein the antibody is produced by screening a Fab expression library.

70. The antibody of claim 10, wherein the antibody is produced by screening a recombinant immunoglobulin library.

71. A method for detecting a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO: 3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 and SEQ ID NO:10, in a sample, comprising the steps of:

a) incubating the antibody of claim 10 with a sample under conditions to allow specific binding of the antibody and the polypeptide; and

b) detecting specific binding, wherein specific binding indicates the presence of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO: 3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 and SEQ ID NO:10, in the sample.

72. A method of purifying a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO: 3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 and SEQ ID NO:10, from a sample, the method comprising:

a) incubating the antibody of claim 10 with a sample under conditions to allow specific binding of the antibody and the polypeptide; and

b) separating the antibody from the sample and obtaining the purified polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO: 3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 and SEQ ID NO:10.

73. A microarray wherein at least one element of the microarray is a polynucleotide of claim 12.

74. A method for generating a transcript image of a sample which contains polynucleotides, the method comprising the steps of:

a) labeling the polynucleotides of the sample,

b) contacting the elements of the microarray of claim 73 with the labeled polynucleotides of the sample under conditions suitable for the formation of a hybridization complex, and

c) quantifying the expression of the polynucleotides in the sample.

75. An array comprising different nucleotide molecules affixed in distinct physical locations on a solid substrate, wherein at least one of said nucleotide molecules comprises a first oligonucleotide or polynucleotide sequence specifically hybridizable with at least 30 contiguous nucleotides of a target polynucleotide, said target polynucleotide having a sequence of claim 11.

76. An array of claim 75, wherein said first oligonucleotide or polynucleotide sequence is completely complementary to at least 30 contiguous nucleotides of said target polynucleotide.

77. An array of claim 75, wherein said first oligonucleotide or polynucleotide sequence is completely complementary to at least 60 contiguous nucleotides of said target polynucleotide.

78. An array of claim 75, which is a microarray.

79. An array of claim 75, further comprising said target polynucleotide hybridized to said first oligonucleotide or polynucleotide.

80. An array of claim 75, wherein a linker joins at least one of said nucleotide molecules to said solid substrate.

81. An array of claim 75, wherein each distinct physical location on the substrate contains multiple nucleotide molecules having the same sequence, and each distinct physical location on the substrate contains nucleotide molecules having a sequence which differs from the sequence of nucleotide molecules at another physical location on the substrate.

Docket No.: PF-0715 USN

DECLARATION AND POWER OF ATTORNEY FOR UNITED STATES PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name, and

I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if more than one name is listed below) of the subject matter which is claimed and for which a United States patent is sought on the invention entitled

HUMAN IMMUNE RESPONSE MOLECULES

the specification of which:

/X/ is attached hereto.

/___/ was filed on _____ as application Serial No. _____ and if this box contains an X /___/, was amended on _____.

/X___/ was filed as Patent Cooperation Treaty international application No. PCT/US00/18505 on July 6, 2000, if this box contains an X /_/, was amended on under Patent Cooperation Treaty Article 19 on _____ 2002, and if this box contains an X /_/, was amended on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge my duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim the benefit under Title 35, United States Code, §119 or §365(a)-(b) of any foreign application(s) for patent or inventor's certificate indicated below and of any Patent Cooperation Treaty international applications(s) designating at least one country other than the United States indicated below and have also identified below any foreign application(s) for patent or inventor's certificate and Patent Cooperation Treaty international application(s) designating at least one country other than the United States for the same subject matter and having a filing date before that of the application for said subject matter the priority of which is claimed:

Country	Number	Filing Date	Priority Claimed
_____	_____	_____	/_/ Yes /_/ No
_____	_____	_____	/_/ Yes /_/ No

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

Application Serial No.	Filed	Status (Pending, Abandoned, Patented)
60/142,572	July 6, 1999	Expired
60/153,170	September 9, 1999	Expired

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in said prior application(s) in the manner required by the first paragraph of Title 35, United States Code §112, I acknowledge my duty to disclose material information as defined in Title 37 Code of Federal Regulations, §1.56(a) which occurred between the filing date(s) of the prior application(s) and the national or Patent Cooperation Treaty international filing date of this application:

Application Serial No.	Filed	Status (Pending, Abandoned, Patented)

I hereby appoint the following:

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respectively and individually, as my patent attorneys and/or agents, with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith. Please address all communications to:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

1-00 10033089-040302

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        290                 295                 300
Ser Arg Asp Arg Glu Lys Thr Lys Ser Leu Glu Ile Thr Gly Glu
    305                 310                 315
Arg Lys Ser Arg Ile Asp Gln Leu Lys Arg Gly Glu Pro Ser Arg
                320                 325                 330
Ser Thr Ser Ser Asp Arg Gln Asp Ser Arg Ser His Ser Ser Arg
            335                 340                 345
Arg Ser Ser Pro Glu Ser Asp Arg Gln Val His Ser Arg Ser Gly
        350                 355                 360
Ser Phe Asp Ser Arg Asp Arg Leu Gln Glu Arg Asp Arg Tyr Glu
    365                 370                 375
His Asp Arg Glu Arg Glu Arg Glu Arg Arg Asp Thr Arg Gln Arg
                380                 385                 390
Glu Trp Asp Arg Asp Ala Asp Lys Asp Trp Pro Arg Asn Arg Asp
            395                 400                 405
Arg Asp Arg Leu Arg Glu Arg Glu Arg Glu Arg Glu Arg Asp Lys
        410                 415                 420
Arg Arg Asp Leu Asp Arg Glu Arg Glu Arg Leu Ile Ser Asp Ser
    425                 430                 435
Val Glu Arg Asp Arg Asp Arg Asp Arg Asp Arg Thr Phe Glu Ser
                440                 445                 450
Ser Gln Ile Glu Ser Val Lys Arg Cys Glu Ala Lys Leu Glu Gly
            455                 460                 465
Glu His Glu Arg Asp Leu Glu Ser Thr Ser Arg Asp Ser Leu Ala
        470                 475                 480
Leu Asp Lys Glu Arg Met Asp Lys Asp Leu Gly Ser Val Gln Gly
    485                 490                 495
Phe Glu Glu Thr Asn Lys Ser Glu Arg Thr Glu Ser Leu Glu Gly
                500                 505                 510
Asp Asp Glu Ser Lys Leu Asp Asp Ala His Ser Leu Gly Ser Gly
            515                 520                 525
Ala Gly Glu Gly Tyr Glu Pro Ile Ser Asp Asp Glu Leu Asp Glu
        530                 535                 540
Ile Leu Ala Gly Asp Ala Glu Lys Arg Glu Asp Gln Gln Asp Glu
    545                 550                 555
Glu Lys Met Pro Asp Pro Leu Asp Val Ile Asp Val Asp Trp Ser
                560                 565                 570
Gly Leu Met Pro Lys His Pro Lys Glu Pro Arg Glu Pro Gly Ala
            575                 580                 585
Ala Leu Leu Lys Phe Thr Pro Gly Ala Val Met Leu Arg Val Gly
        590                 595                 600
Ile Ser Lys Lys Leu Ala Gly Ser Glu Leu Phe Ala Lys Val Lys
    605                 610                 615
Glu Thr Cys Gln Arg Leu Leu Glu Lys Pro Lys Asp Ala Asp Asn
                620                 625                 630
Leu Phe Glu His Glu Leu Gly Ala Leu Asn Met Ala Ala Leu Leu
            635                 640                 645
Arg Lys Glu Glu Arg Ala Ser Leu Leu Ser Asn Leu Gly Pro Cys
        650                 655                 660
Cys Lys Ala Leu Cys Phe Arg Arg Asp Ser Ala Ile Arg Lys Gln
    665                 670                 675
Leu Val Lys Asn Glu Lys Gly Thr Ile Arg Gln Ala Tyr Thr Ser
                680                 685                 690
Ala Pro Met Val Asp Asn Glu Leu Leu Arg Leu Ser Leu Arg Leu
            695                 700                 705
Phe Lys Arg Lys Thr Thr Cys His Ala Pro Gly His Glu Lys Thr
        710                 715                 720
Glu Asp Asn Lys Leu Ser Gln Ser Ser Ile Gln Gln Glu Leu Cys
    725                 730                 735
Val Ser
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<210> 2
<211> 319
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 2378306CD1

<400> 2
Met Arg Leu Ala Thr Lys Gly Gln Asp Ile Gln Glu Cys Thr Thr
1 5 10 15
Gln Ile Gln Tyr Leu Lys Gln Val Gln Gln Pro Ser Val Ala Gln
20 25 30
Leu Arg Ser Thr Met Val Asp Pro Ala Ile Asn Leu Phe Phe Leu
35 40 45
Lys Met Lys Gly Glu Leu Glu Gln Thr Lys Asp Lys Leu Glu Gln
50 55 60
Ala Gln Asn Glu Leu Ser Ala Trp Lys Phe Thr Pro Asp Ser Gln
65 70 75
Thr Gly Lys Lys Leu Met Ala Lys Cys Arg Met Leu Ile Gln Glu
80 85 90
Asn Gln Glu Leu Gly Arg Gln Leu Ser Gln Gly Arg Ile Ala Gln
95 100 105
Leu Glu Ala Glu Leu Ala Leu Gln Lys Lys Tyr Ser Glu Glu Leu
110 115 120
Lys Ser Ser Gln Asp Glu Leu Asn Asp Phe Ile Ile Gln Leu Asp
125 130 135
Glu Glu Val Glu Gly Met Gln Ser Thr Ile Leu Val Leu Gln Gln
140 145 150
Gln Leu Lys Glu Thr Arg Gln Gln Leu Ala Gln Tyr Gln Gln Gln
155 160 165
Gln Ser Gln Ala Ser Ala Pro Ser Thr Ser Arg Thr Thr Ala Ser
170 175 180
Glu Pro Val Glu Gln Ser Glu Ala Thr Ser Lys Asp Cys Ser Arg
185 190 195
Leu Thr Asn Gly Pro Ser Asn Gly Ser Ser Ser Arg Gln Arg Thr
200 205 210
Ser Gly Ser Gly Phe His Arg Glu Gly Asn Thr Thr Glu Asp Asp
215 220 225
Phe Pro Ser Ser Pro Gly Asn Gly Asn Lys Ser Ser Asn Ser Ser
230 235 240
Glu Glu Arg Thr Gly Arg Gly Gly Ser Gly Tyr Val Asn Gln Leu
245 250 255
Ser Ala Gly Tyr Glu Ser Val Asp Ser Pro Thr Gly Ser Glu Asn
260 265 270
Ser Leu Thr His Gln Ser Asn Asp Thr Asp Ser Ser His Asp Pro
275 280 285
Gln Glu Glu Lys Ala Val Ser Gly Lys Gly Asn Arg Thr Val Gly
290 295 300
Ser Arg His Val Gln Asn Gly Leu Asp Ser Ser Val Asn Val Gln
305 310 315
Gly Ser Val Leu

<210> 3
<211> 131
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 3706232CD1

<400> 3
Met Arg Leu Phe Leu Trp Asn Ala Val Leu Thr Leu Phe Val Thr
1 5 10 15
Ser Leu Ile Gly Ala Leu Ile Pro Glu Pro Glu Val Lys Ile Glu

```
                    20                  25                  30
Val Leu Gln Lys Pro Phe Ile Cys His Arg Lys Thr Lys Gly Gly
                35                  40                  45
Asp Leu Met Leu Val His Tyr Glu Gly Tyr Leu Glu Lys Asp Gly
            50                  55                  60
Ser Leu Phe His Ser Thr His Lys His Asn Asn Gly Gln Pro Ile
        65                  70                  75
Trp Phe Thr Leu Gly Ile Leu Glu Ala Leu Lys Gly Trp Asp Gln
                80                  85                  90
Gly Leu Lys Gly Met Cys Val Gly Glu Lys Arg Lys Leu Ile Ile
            95                  100                 105
Pro Pro Ala Leu Gly Tyr Gly Lys Glu Gly Lys Glu Leu Ser Ala
        110                 115                 120
Thr His Pro Cys Arg Arg Met Phe Lys Gln Ile
    125                 130
<210> 4
<211> 642
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 259836CD1

<400> 4
Met Ile Pro Asn Gly Tyr Leu Met Phe Glu Asp Glu Asn Phe Ile
 1               5                  10                  15
Glu Ser Ser Val Ala Lys Leu Asn Ala Leu Arg Lys Ser Gly Gln
            20                  25                  30
Phe Cys Asp Val Arg Leu Gln Val Cys Gly His Glu Met Leu Ala
        35                  40                  45
His Arg Ala Val Leu Ala Cys Cys Ser Pro Tyr Leu Phe Glu Ile
    50                  55                  60
Phe Asn Ser Asp Ser Asp Pro His Gly Ile Ser His Val Lys Phe
65                  70                  75
Asp Asp Leu Asn Pro Glu Ala Val Glu Val Leu Leu Asn Tyr Ala
                80                  85                  90
Tyr Thr Ala Gln Leu Lys Ala Asp Lys Glu Leu Val Lys Asp Val
            95                  100                 105
Tyr Ser Ala Ala Lys Lys Leu Lys Met Asp Arg Val Lys Gln Val
        110                 115                 120
Cys Gly Asp Tyr Leu Leu Ser Arg Met Asp Val Thr Ser Cys Ile
    125                 130                 135
Ser Tyr Arg Asn Phe Ala Ser Cys Met Gly Asp Ser Arg Leu Leu
140                 145                 150
Asn Lys Val Asp Ala Tyr Ile Gln Glu His Leu Leu Gln Ile Ser
            155                 160                 165
Glu Glu Glu Glu Phe Leu Lys Leu Pro Arg Leu Lys Leu Glu Val
        170                 175                 180
Met Leu Glu Asp Asn Val Cys Leu Pro Ser Asn Gly Lys Leu Tyr
    185                 190                 195
Thr Lys Val Ile Asn Trp Val Gln Arg Ser Ile Trp Glu Asn Gly
200                 205                 210
Asp Ser Leu Glu Glu Leu Met Glu Glu Val Gln Thr Leu Tyr Tyr
            215                 220                 225
Ser Ala Asp His Lys Leu Leu Asp Gly Asn Leu Leu Asp Gly Gln
        230                 235                 240
Ala Glu Val Phe Gly Ser Asp Asp Asp His Ile Gln Phe Val Gln
    245                 250                 255
Lys Lys Pro Pro Arg Glu Asn Gly His Lys Gln Ile Ser Ser Ser
260                 265                 270
Ser Thr Gly Cys Leu Ser Ser Pro Asn Ala Thr Val Gln Ser Pro
            275                 280                 285
Lys His Glu Trp Lys Ile Val Ala Ser Glu Lys Thr Ser Asn Asn
        290                 295                 300
Thr Tyr Leu Cys Leu Ala Val Leu Asp Gly Ile Phe Cys Val Ile
305                 310                 315
```

```
Phe Leu His Gly Arg Asn Ser Pro Gln Ser Ser Pro Thr Ser Thr
                320                 325                 330
Pro Lys Leu Ser Lys Ser Leu Ser Phe Glu Met Gln Gln Asp Glu
                335                 340                 345
Leu Ile Glu Lys Pro Met Ser Pro Met Gln Tyr Ala Arg Ser Gly
                350                 355                 360
Leu Gly Thr Ala Glu Met Asn Gly Lys Leu Ile Ala Ala Gly Gly
                365                 370                 375
Tyr Asn Arg Glu Glu Cys Leu Arg Thr Val Glu Cys Tyr Asn Pro
                380                 385                 390
His Thr Asp His Trp Ser Phe Leu Ala Pro Met Arg Thr Pro Arg
                395                 400                 405
Ala Arg Phe Gln Met Ala Val Leu Met Gly Gln Leu Tyr Val Val
                410                 415                 420
Gly Gly Ser Asn Gly His Ser Asp Asp Leu Ser Cys Gly Glu Met
                425                 430                 435
Tyr Asp Ser Asn Ile Asp Asp Trp Ile Pro Val Pro Glu Leu Arg
                440                 445                 450
Thr Asn Arg Cys Asn Ala Gly Val Cys Ala Leu Asn Gly Lys Leu
                455                 460                 465
Tyr Ile Val Gly Gly Ser Asp Pro Tyr Gly Gln Lys Gly Leu Lys
                470                 475                 480
Asn Cys Asp Val Phe Asp Pro Val Thr Lys Leu Trp Thr Ser Cys
                485                 490                 495
Ala Pro Leu Asn Ile Arg Arg His Gln Ser Ala Val Cys Glu Leu
                500                 505                 510
Gly Gly Tyr Leu Tyr Ile Ile Gly Gly Ala Glu Ser Trp Asn Cys
                515                 520                 525
Leu Asn Thr Val Glu Arg Tyr Asn Pro Glu Asn Asn Thr Trp Thr
                530                 535                 540
Leu Ile Ala Pro Met Asn Val Ala Arg Arg Gly Ala Gly Val Ala
                545                 550                 555
Val Leu Asn Gly Lys Leu Phe Val Cys Gly Gly Phe Asp Gly Ser
                560                 565                 570
His Ala Ile Ser Cys Val Glu Met Tyr Asp Pro Thr Arg Asn Glu
                575                 580                 585
Trp Lys Met Met Gly Asn Met Thr Ser Pro Arg Ser Asn Ala Gly
                590                 595                 600
Ile Ala Thr Val Gly Asn Thr Ile Tyr Ala Val Gly Gly Phe Asp
                605                 610                 615
Gly Asn Glu Phe Leu Asn Thr Val Glu Val Tyr Asn Leu Glu Ser
                620                 625                 630
Asn Glu Trp Ser Pro Tyr Thr Lys Ile Phe Gln Phe
                635                 640
<210> 5
<211> 349
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 893681CD1

<400> 5
Met Glu Thr Pro Pro Val Asn Thr Ile Gly Glu Lys Asp Thr Ser
  1               5                  10                  15
Gln Pro Gln Gln Glu Trp Glu Lys Asn Leu Arg Glu Asn Leu Asp
                20                  25                  30
Ser Val Ile Gln Ile Arg Gln Gln Pro Arg Asp Pro Pro Thr Glu
                35                  40                  45
Thr Leu Glu Leu Glu Val Ser Pro Asp Pro Ala Ser Gln Ile Leu
                50                  55                  60
Glu His Thr Gln Gly Ala Glu Lys Leu Val Ala Glu Leu Glu Gly
                65                  70                  75
Asp Ser His Lys Ser His Gly Ser Thr Ser Gln Met Pro Glu Ala
                80                  85                  90
Leu Gln Ala Ser Asp Leu Trp Tyr Cys Pro Asp Gly Ser Phe Val
```

```
                 95                  100                 105
Lys Lys Ile Val Ile Arg Gly His Gly Leu Asp Lys Pro Lys Leu
                110                 115                 120
Gly Ser Cys Cys Arg Val Leu Ala Leu Gly Phe Pro Phe Gly Ser
                125                 130                 135
Gly Pro Pro Glu Gly Trp Thr Glu Leu Thr Met Gly Val Gly Pro
                140                 145                 150
Trp Arg Glu Glu Thr Trp Gly Glu Leu Ile Glu Lys Cys Leu Glu
                155                 160                 165
Ser Met Cys Gln Gly Glu Glu Ala Glu Leu Gln Leu Pro Gly His
                170                 175                 180
Ser Gly Pro Pro Val Arg Leu Thr Leu Ala Ser Phe Thr Gln Gly
                185                 190                 195
Arg Asp Ser Trp Glu Leu Glu Thr Ser Glu Lys Glu Ala Leu Ala
                200                 205                 210
Arg Glu Glu Arg Ala Arg Gly Thr Glu Leu Phe Arg Ala Gly Asn
                215                 220                 225
Pro Glu Gly Ala Ala Arg Cys Tyr Gly Arg Ala Leu Arg Leu Leu
                230                 235                 240
Leu Thr Leu Pro Pro Pro Gly Pro Pro Glu Arg Thr Val Leu His
                245                 250                 255
Ala Asn Leu Ala Ala Cys Gln Leu Leu Leu Gly Gln Pro Gln Leu
                260                 265                 270
Ala Ala Gln Ser Cys Asp Arg Val Leu Glu Arg Glu Pro Gly His
                275                 280                 285
Leu Lys Ala Leu Tyr Arg Arg Gly Val Ala Gln Ala Ala Leu Gly
                290                 295                 300
Asn Leu Glu Lys Ala Thr Ala Asp Leu Lys Lys Val Leu Ala Ile
                305                 310                 315
Asp Pro Lys Asn Arg Ala Ala Gln Glu Glu Leu Gly Lys Val Val
                320                 325                 330
Ile Gln Gly Lys Asn Gln Asp Ala Gly Leu Ala Gln Gly Leu Arg
                335                 340                 345
Lys Met Phe Gly
```

<210> 6
<211> 300
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 2109468CD1

<400> 6

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Met Ala Glu Ser Glu Asp Arg Ser Leu Arg Ile Val Leu Val Gly
  1                 5                  10                  15
Lys Thr Gly Ser Gly Lys Ser Ala Thr Ala Asn Thr Ile Leu Gly
                 20                  25                  30
Glu Glu Ile Phe Asp Ser Arg Ile Ala Ala Gln Ala Val Thr Lys
                 35                  40                  45
Asn Cys Gln Lys Ala Ser Arg Glu Trp Gln Gly Arg Asp Leu Leu
                 50                  55                  60
Val Val Asp Thr Pro Gly Leu Phe Asp Thr Lys Glu Ser Leu Asp
                 65                  70                  75
Thr Thr Cys Lys Glu Ile Ser Arg Cys Ile Ile Ser Ser Cys Pro
                 80                  85                  90
Gly Pro His Ala Ile Val Leu Val Leu Leu Leu Gly Arg Tyr Thr
                 95                 100                 105
Glu Glu Glu Gln Lys Thr Val Ala Leu Ile Lys Ala Val Phe Gly
                110                 115                 120
Lys Ser Ala Met Lys His Met Val Ile Leu Phe Thr Arg Lys Glu
                125                 130                 135
Glu Leu Glu Gly Gln Ser Phe His Asp Phe Ile Ala Asp Ala Asp
                140                 145                 150
Val Gly Leu Lys Ser Ile Val Lys Glu Cys Gly Asn Arg Cys Cys
                155                 160                 165
```

```
Ala Phe Ser Asn Ser Lys Lys Thr Ser Lys Ala Glu Lys Glu Ser
                170                 175                 180
Gln Val Gln Glu Leu Val Glu Leu Ile Glu Lys Met Val Gln Cys
                185                 190                 195
Asn Glu Gly Ala Tyr Phe Ser Asp Asp Ile Tyr Lys Asp Thr Glu
                200                 205                 210
Glu Arg Leu Lys Gln Arg Glu Glu Val Leu Arg Lys Ile Tyr Thr
                215                 220                 225
Asp Gln Leu Asn Glu Glu Ile Lys Leu Val Glu Glu Asp Lys His
                230                 235                 240
Lys Ser Glu Glu Glu Lys Glu Lys Glu Ile Lys Leu Leu Lys Leu
                245                 250                 255
Lys Tyr Asp Glu Lys Ile Lys Asn Ile Arg Glu Glu Ala Glu Arg
                260                 265                 270
Asn Ile Phe Lys Asp Val Phe Asn Arg Ile Trp Lys Met Leu Ser
                275                 280                 285
Glu Ile Trp His Arg Phe Leu Ser Lys Cys Lys Phe Tyr Ser Ser
                290                 295                 300

<210> 7
<211> 118
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 2223341CD1

<400> 7
Met Ser Cys Thr Ile Glu Lys Ala Leu Ala Asp Ala Lys Ala Leu
  1               5                  10                  15
Val Glu Arg Leu Arg Asp His Asp Asp Ala Ala Glu Ser Leu Ile
                20                  25                  30
Glu Gln Thr Thr Ala Leu Asn Lys Arg Val Glu Ala Met Lys Gln
                35                  40                  45
Tyr Gln Glu Glu Ile Gln Glu Leu Asn Glu Val Ala Arg His Arg
                50                  55                  60
Pro Arg Ser Thr Leu Val Met Gly Ile Gln Gln Glu Asn Arg Gln
                65                  70                  75
Ile Arg Glu Leu Gln Gln Glu Asn Lys Glu Leu Arg Thr Ser Leu
                80                  85                  90
Glu Glu His Gln Ser Ala Leu Glu Leu Ile Met Ser Lys Tyr Arg
                95                 100                 105
Glu Gln Met Phe Arg Leu Leu Met Ala Ser Lys Lys Arg
               110                 115
<210> 8
<211> 313
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 2291926CD1

<400> 8
Met His Leu Ala Leu Thr Thr Val Leu Leu Trp Ala Trp Gly Ser
  1               5                  10                  15
Leu Gln Ala Phe Glu Ile Val Glu Lys Glu Asn Ile Phe Gln Arg
                20                  25                  30
Thr Pro Cys Pro Ala Phe Leu Met Phe Glu Asn Ala Ala Tyr Leu
                35                  40                  45
Ala Asp Met Ser Phe Glu Leu Pro Cys His Cys Lys Pro Glu Glu
                50                  55                  60
Val Pro Ala Val Val Trp Phe Tyr Gln Lys His Leu Gly Ser Ser
                65                  70                  75
His Thr Lys Val Leu Thr Asp Phe Asp Gly Arg Val Leu Thr Glu
                80                  85                  90
```

```
Ala Ala Gln Val Arg Val Gly Ser Asp Met Leu Thr Arg Phe Ser
                 95                  100                 105
Ile Arg Met Phe Ser Leu Leu Val Phe Arg Ala Gln Ser Glu Asp
            110                 115                 120
Ser Gly Leu Tyr Phe Cys Gly Thr Arg Lys Gly Asp Tyr Phe Tyr
        125                 130                 135
Ala Tyr Asp Val Asp Ile Gln Asn Ser Glu Gly Met Val Ala Thr
    140                 145                 150
Phe Gln Asp Glu Gly Gln Glu Pro Phe Ala Asp Glu Tyr Tyr Gly
155                 160                 165
His Leu His Val Phe Thr Thr Phe Trp Glu Trp Thr Pro Cys Asp
                170                 175                 180
Arg Cys Gly Val Arg Gly Glu Gln Trp Arg Ile Gly Leu Cys Tyr
            185                 190                 195
Leu Gln Ser Pro Asp Leu Ser Pro Arg Tyr Leu Lys Ala Val Pro
        200                 205                 210
Asp Val Val Ser Cys Gly Ser Arg Ala Val Pro Arg Lys Leu Arg
    215                 220                 225
Thr Lys Ala Arg Asp His Thr Pro Glu Val Leu Val Arg Ser Cys
230                 235                 240
Leu Val Pro Cys Glu Lys Thr Lys Thr Ile Arg Glu Gly Val Leu
                245                 250                 255
Ala Ile Ile Asn Tyr Val Ser Lys Val Gly Ser Arg Pro Leu Gly
            260                 265                 270
Ala Pro Gly Ala His Ser Val Pro Pro Ala Glu Thr Gly Pro Trp
        275                 280                 285
Thr His His Leu Leu Ser Trp Ala Arg Gln Ser Met Gln Trp Pro
    290                 295                 300
Gly Thr Lys Thr Ala Ser Thr Ser Thr Ala His Ser Thr
305                 310
```

<210> 9
<211> 139
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 2362126CD1

<400> 9

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Met Glu Phe Phe Cys Trp Phe Trp Gly Phe Trp Ser Trp Leu Leu
  1                 5                  10                  15
Asn Met Ile Arg Pro Gln Asn Ala Lys Asp Ser Thr Ser Asn Ser
            20                  25                  30
Met Glu Asn Thr Asp Ser Pro Trp Cys Glu Leu Phe Lys Glu Leu
        35                  40                  45
Tyr Lys Ile Asn Ala Phe Asp Thr Pro Asp Ser Leu Leu Met Arg
    50                  55                  60
Gly Lys Glu Phe Ser Asp Pro Ile His His Thr Phe Asp His Met
65                  70                  75
Trp Arg Thr Lys Glu His Asn Glu Ala Gly Trp Leu Leu Leu Ser
                80                  85                  90
Ser Val Asp Lys Val Met Lys Glu Asn Asp Glu Leu Gly Asp Ser
            95                  100                 105
Ile Ser Gln Leu Gln Lys Gln Ile Leu Ser Leu Lys Ser Ala Lys
        110                 115                 120
Ile Ala Leu Ser Glu Ser Leu Ile Ser Cys Arg Glu Arg Thr Glu
    125                 130                 135
Ile Val Glu Lys
```

<210> 10
<211> 565
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature

<223> Incyte ID No: 5325511CD1

<400> 10

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Met Asn Val Ile Asp His Val Arg Asp Met Ala Ala Ala Gly Leu
  1               5                  10                  15
His Ser Asn Val Arg Leu Leu Ser Ser Leu Leu Leu Thr Met Ser
             20                  25                  30
Asn Asn Asn Pro Glu Leu Phe Ser Pro Pro Gln Lys Tyr Gln Leu
         35                  40                  45
Leu Val Tyr His Ala Asp Ser Leu Phe His Asp Lys Glu Tyr Arg
     50                  55                  60
Asn Ala Val Ser Lys Tyr Thr Met Ala Leu Gln Gln Lys Lys Ala
 65                  70                  75
Leu Ser Lys Thr Ser Lys Val Arg Pro Ser Thr Gly Asn Ser Ala
             80                  85                  90
Ser Thr Pro Gln Ser Gln Cys Leu Pro Ser Glu Ile Glu Val Lys
         95                 100                 105
Tyr Lys Met Ala Glu Cys Tyr Thr Met Leu Lys Gln Asp Lys Asp
    110                 115                 120
Ala Ile Ala Ile Leu Asp Gly Ile Pro Ser Arg Gln Arg Thr Pro
    125                 130                 135
Lys Ile Asn Met Met Leu Ala Asn Leu Tyr Lys Lys Ala Gly Gln
    140                 145                 150
Glu Arg Pro Ser Val Thr Ser Tyr Lys Glu Val Leu Arg Gln Cys
155                 160                 165
Pro Leu Ala Leu Asp Ala Ile Leu Gly Leu Leu Ser Leu Ser Val
    170                 175                 180
Lys Gly Ala Glu Val Ala Ser Met Thr Met Asn Val Ile Gln Thr
    185                 190                 195
Val Pro Asn Leu Asp Trp Leu Ser Val Trp Ile Lys Ala Tyr Ala
    200                 205                 210
Phe Val His Thr Gly Asp Asn Ser Arg Ala Ile Ser Thr Ile Cys
215                 220                 225
Ser Leu Glu Lys Lys Ser Leu Leu Arg Asp Asn Val Asp Leu Leu
    230                 235                 240
Gly Ser Leu Ala Asp Leu Tyr Phe Arg Ala Gly Asp Asn Lys Asn
    245                 250                 255
Ser Val Leu Lys Phe Glu Gln Ala Gln Met Leu Asp Pro Tyr Leu
    260                 265                 270
Ile Lys Gly Met Asp Val Tyr Gly Tyr Leu Leu Ala Arg Glu Gly
    275                 280                 285
Arg Leu Glu Asp Val Glu Asn Leu Gly Cys Arg Leu Phe Asn Ile
    290                 295                 300
Ser Asp Gln His Ala Glu Pro Trp Val Val Ser Gly Cys His Ser
305                 310                 315
Phe Tyr Ser Lys Arg Tyr Ser Arg Ala Leu Tyr Leu Gly Ala Lys
    320                 325                 330
Ala Ile Gln Leu Asn Ser Asn Ser Val Gln Ala Leu Leu Leu Lys
    335                 340                 345
Gly Ala Ala Leu Arg Asn Met Gly Arg Val Gln Glu Ala Ile Ile
    350                 355                 360
His Phe Arg Glu Ala Ile Arg Leu Ala Pro Cys Arg Leu Asp Cys
365                 370                 375
Tyr Glu Gly Leu Ile Glu Cys Tyr Leu Ala Ser Asn Ser Ile Arg
    380                 385                 390
Glu Ala Met Val Met Ala Asn Asn Val Tyr Lys Thr Leu Gly Ala
    395                 400                 405
Asn Ala Gln Thr Leu Thr Leu Leu Ala Thr Val Cys Leu Glu Asp
    410                 415                 420
Pro Val Thr Gln Glu Lys Ala Lys Thr Leu Leu Asp Lys Ala Leu
425                 430                 435
Thr Gln Arg Pro Asp Tyr Ile Lys Ala Val Val Lys Lys Ala Glu
    440                 445                 450
Leu Leu Ser Arg Glu Gln Lys Tyr Glu Asp Gly Ile Ala Leu Leu
    455                 460                 465
Arg Asn Ala Leu Ala Asn Gln Ser Asp Cys Val Leu His Arg Ile
    470                 475                 480
```

Leu Gly Asp Phe Leu Val Ala Val Asn Glu Tyr Gln Glu Ala Met
485 490 495
Asp Gln Tyr Ser Ile Ala Leu Ser Leu Asp Pro Asn Asp Gln Lys
500 505 510
Ser Leu Glu Gly Met Gln Lys Met Glu Lys Glu Glu Ser Pro Thr
515 520 525
Asp Ala Thr Gln Glu Glu Asp Val Asp Asp Met Glu Gly Ser Gly
530 535 540
Glu Glu Gly Asp Leu Glu Gly Ser Asp Ser Glu Ala Ala Gln Trp
545 550 555
Ala Asp Gln Glu Gln Trp Phe Gly Met Gln
560 565

<210> 11
<211> 3360
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 089071CB1

<400> 11
gataaagaac gagaacgcca aagggattgg gaagacaaag acaaaggacg agatgaccgc 60
agagaaaagc gagaagagat ccgagaagat aggaatccaa gagatggaca tgatgaaaga 120
aaatcaaaga agcgctatag aaatgaaggg agtcccagcc ctagacagtc cccgaagcgc 180
cggcgtgaac attctccgga cagtgatgcc tacaacagtg gagatgataa aaatgaaaaa 240
cacagactct tgagccaagt tgtacgacct caagaatctc gttctcttag tccctcgcac 300
ctcacagaag acagacaggg tagatggaaa gaggaggatc gtaaaccaga aaggaaagag 360
agttcaaggc gctacgaaga acaggaactc aaggagaaag tttcttctgt agataaacag 420
agagaacaga cagaaatcct ggaaagctca agaatgcgtg cacaggacat tataggacac 480
caccagtctg aagatcgaga gacatctgat cgagctcatg atgaaaacaa gaagaaagca 540
aaaattcaaa agaaaccaat taagaaaaag aaagaggatg atgttggaat agagaggggt 600
aacatagaga caacatctga agatggtcaa gtattttcac caaaaaaagg acagaaaaag 660
aaaagcattg aaaaaaaacg taaaaaatcc aaaggtgatt ctgatatttc tgatgaagaa 720
gcagtccagc aaagtaagaa gaaaagaggc ccacggactc cccctataac aactaaagag 780
gaattggttg aaatgtgcag tggtaagaat ggtattctag aggactccca gaaaaaagaa 840
gatacagcat tcagtgactg gtctgatgag gatgtccctg accgtacaga ggtgacagaa 900
gcagagcata ctgccaccgc cacgactcct ggtagtaccc cttctcctct atcttctctt 960
cttcctcctc caccgcctgt ggctactgcc actgctacaa ctgtgcctgc aactcttgct 1020
gccactactg ctgctgccgc cacctctttc agcacatctg ccatcactat ttccacctct 1080
gccacccccca ccaataccac caataatact tttgccaatg aagactcaca cagaaaatgc 1140
cacagaacac gagtagaaaa agtagagacg cctcacgtga ctatagaaga tgcacagcat 1200
cgcaagccta tggatcaaaa gaggagcagc agcctcggga gcaatcggag taaccgtagt 1260
catacgtctg gtcgtcttcg ctccccatcc aatgattcag cccatcgaag tggagatgac 1320
caaagtggtc gaaagagagt actgcacagt ggctcaagag atagagaaaa aacaaaaagc 1380
ctggaaatca caggagagag aaaatctagg attgatcagt taaagcgtgg agaacccagt 1440
cgaagtactt cttcagatcg ccaggattca agaagccata gttcaagaag aagttctcca 1500
gagtcagatc gacaggtcca ttcaagatct gggtcatttg atagcagaga caggcttcaa 1560
gaacgagatc gatatgaaca cgacagagag cgcgagagag agaggagaga tacgaggcag 1620
agagaatggg accgagatgc tgataaagat tggccacgca acagggatcg agatagattg 1680
cgagaacgag aacgagagag agaacgagac aaaaggagag acttggatag ggaaagagag 1740
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<213> Homo sapiens

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<213> Homo sapiens

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<212> DNA
<213> Homo sapiens

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<213> Homo sapiens

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<223> Incyte ID No: 893681CB1

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<212> DNA
<213> Homo sapiens

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<212> DNA
<213> Homo sapiens

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<221> unsure
<222> 1177
<223> a, t, c, g, or other
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<211> 1673
<212> DNA
<213> Homo sapiens

<220>
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<211> 1100

<212> DNA
<213> Homo sapiens

<220>
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<210> 20
<211> 2827
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